4. RESULTS AND DISCUSSION

The research work carried out to achieve the planned aims and objectives has been described under two sections,

4.1 Anthranilamide-based Factor Xa inhibitors

4.2 Furanopyrimidinone-based Thrombin inhibitors

4.1 Anthranilamide-based Factor Xa inhibitors

The detailed studies of synthesis, biological activity and molecular docking studies and physicochemical properties of anthranilamide scaffold containing derivatives are discussed under following sub-heads:

4.1.1 Synthesis of piperazinyl anthranilamide derivatives

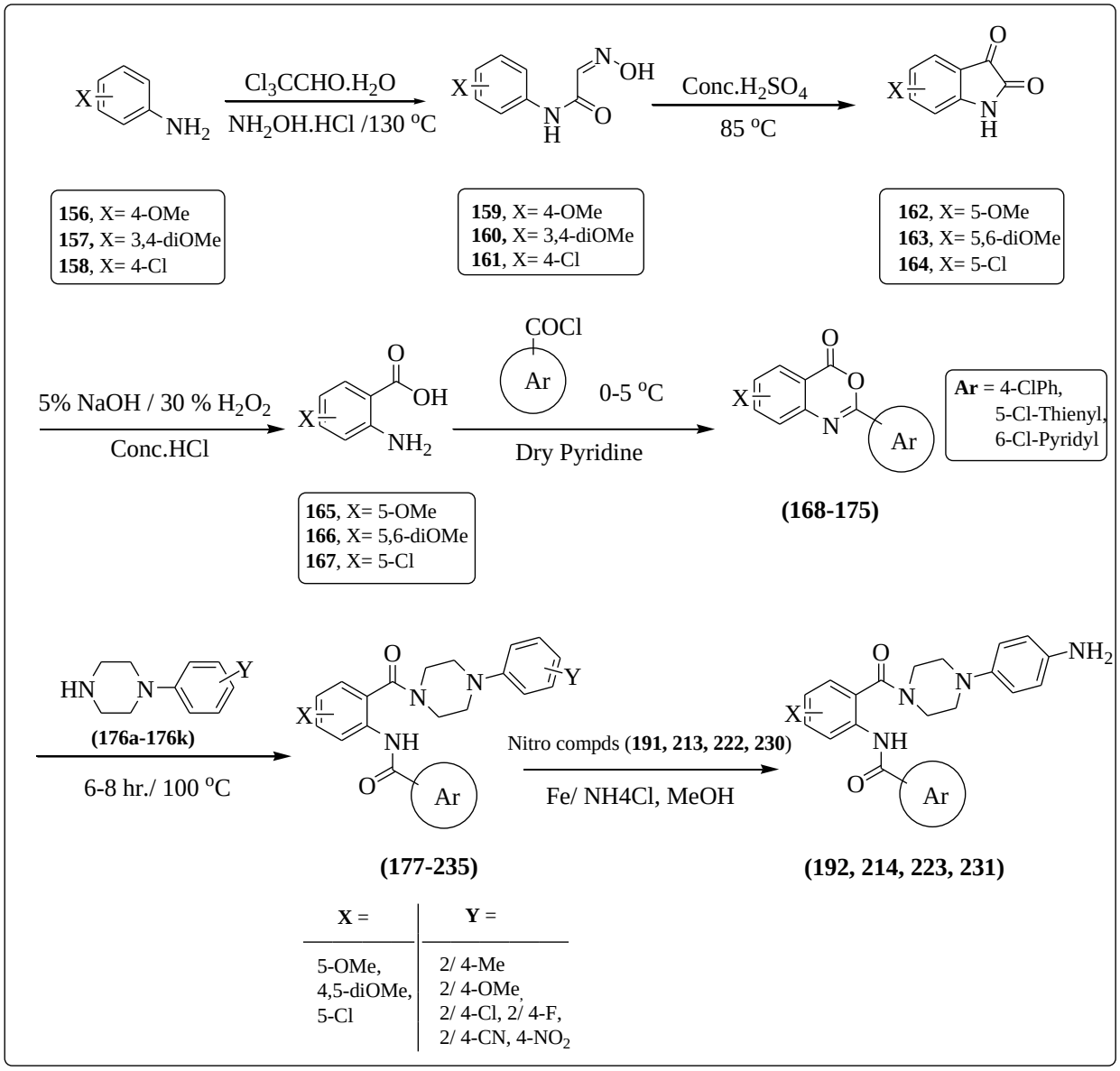
4.1.2 Biological evaluation of target compounds

4.1.3 Molecular docking and simulation studies

4.1.4 Prediction of physicochemical properties

4.1.1 Synthesis of piperazinyl anthranilamide derivatives

The synthetic scheme employed for the designed anthranilamide derivatives is depicted in general **scheme 4.1**. Substituted oximes (**159-161**) were obtained by reacting substituted anilines (**156-158**) with chloral hydrate and hydroxylamine hydrochloride followed by their cyclization into isatin derivatives (**162-164**) using concentrated sulphuric acid. The isatins so obtained were converted into anthranilic acids by alkaline hydrogen peroxide oxidation followed by acidic hydrolysis. The anthranilic acids (**165-167**) were then reacted with different aromatic acid chlorides in presence of dry pyridine affording different 1,3-benzoxazin-4-one analogs (**168-175**). The benzoxazinone intermediates (**168-175**) were finally heated with different piperazine bases offering the target compounds (**177-235**). The nitro derivatives were further reduced to amino analogs (**192, 214, 223** and **231**) by iron powder and ammonium chloride in methanol. All the targeted compounds were purified by column chromatography.



Scheme 4.1

The research work planned for the synthesis of the target compounds ie. substituted anthranilamides are discussed under the following headings:

(i) Synthesis of substituted 2-(hydroxyimino)-*N*-phenylacetamide derivatives (**159-161**)

(ii) Synthesis of substituted indoline-2,3-diones (**162-164**)

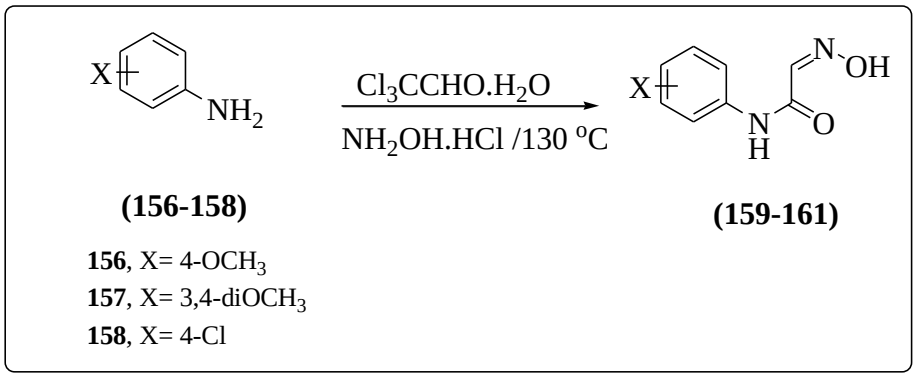
(iii) Synthesis of substituted 2-aminobenzoic acids (**165-167**)

(iv) Synthesis of substituted benz[d][1,3]oxazin-4-one derivatives (**168-175**)

(v) Synthesis of substituted anthranilamides (**177-235**)

4.1.1: Synthesis of substituted 2-(hydroxyimino)-*N*-phenylacetamides (**159-161**)

Commercially available anilines (**156-158**) were heated with a mixture of chloral hydrate and hydroxylamine hydrochloride in aqueous medium to get substituted 2-(hydroxyimino)-*N*-phenylacetamide derivatives (**scheme 4.2**). Infrared (IR) spectra of these compounds displayed presence of characteristic peak of –OH stretching of oxime at 3200-3400 cm^{-1} and C=O stretching of amide at 1650-1670 cm^{-1} and absence of -NH stretching of primary amine.



(156-158)

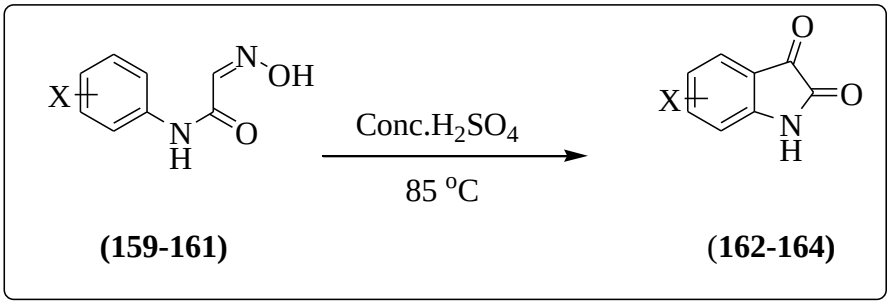
156, X= 4-OCH_3
157, X= 3,4-diOCH_3
158, X= 4-Cl

(159-161)

Scheme: 4.2

4.1.2: Synthesis of substituted indoline-2,3-diones (**162-164**)

2-(Hydroxyimino)-*N*-phenylacetamides (**159-161**) obtained from anilines were then cyclized into substituted indoline-2,3-diones via Sandmeyer isatin synthesis[1] by the action of concentrated sulphuric acid at 85 ^{0}C (**scheme 4.3**). IR spectra of the compounds revealed peaks for C=O stretching at 1750-1740 (ketone C=O) and 1710 cm^{-1} (amide C=O) and disappearance of broad –OH stretching peak at 3400 cm^{-1} .



(159-161)

(162-164)

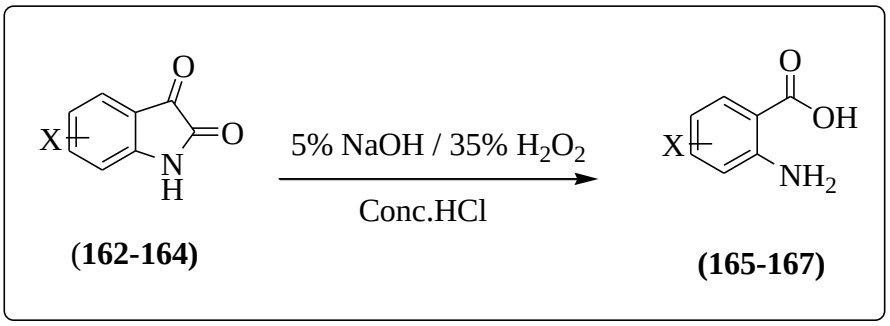
Scheme: 4.3

Table 4.1: Infra-red data of intermediate compounds **(159-167)**

Compound	X	IR bands (cm^{-1})
159	4-OMe	1660 (C=O stretching), 1616, 1562, 1512, 1249, 1002.
160	3,4-DiOMe	3388 (NH stretching), 1656 (C=O stretching), 1622, 1257, 1026.
161	4-Cl	3300 (NH stretching), 1664 (C=O stretching), 1615, 1552, 1248, 1007.
162	5-OMe	1745 (C=O stretching), 1633, 1489, 1032.
163	5,6-DiOMe	3300 (NH stretching), 1758 (C=O stretching), 1714 (C=O stretching), 1622, 1496, 1006.
164	5-Cl	3183, 1755 (C=O stretching), 1709 (C=O stretching), 1615, 1450.
165	5-OMe	3399 and 3311 (NH stretching), 1660 (C=O stretching), 1602, 1030.
166	4,5-DiOMe	3487 and 3372 (NH stretching), 1655 (C=O stretching), 1420, 996.
167	5-Cl	3468 and 3357 (NH stretching), 1675 (C=O stretching), 1238.

4.1.3: Synthesis of substituted 2-amino benzoic acids (**165-167**)

Indoline-2,3-diones were further oxidized to substituted 2-aminobenzoic acids (anthranilic acids) by use of 35% hydrogen peroxide solution in the basic medium followed by acidic hydrolysis[2] (**scheme 4.4**). These anthranilic acids exhibited two peaks of –NH stretching of primary amino groups with broad peak of –OH stretching at 3400-3200 cm^{-1} as well as C=O (carboxyl carbonyl) stretching peak at about 1660-1670 cm^{-1} in their IR spectra.

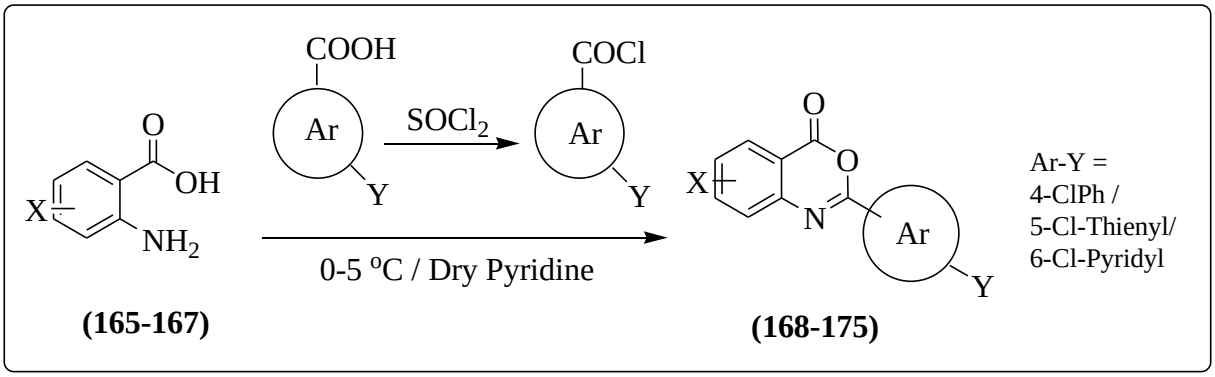


(162-164) **(165-167)**

Scheme: 4.4

4.1.4: Synthesis of substituted benz[*d*][1,3]oxazin-4-one derivatives (168-175)

Benz[*d*][1,3]oxazin-4-one derivatives were synthesized by reacting substituted acid chlorides and 2-amino benzoic acids (anthranilic acids) using dry pyridine base under cold conditions at 0-5 ^0C [3] (**scheme 4.5**). Acid chlorides were prepared from aryl acids by refluxing them with thionyl chloride till completion of the reaction. Presence of characteristic peak of C=O stretching from lactone carbonyl was observed in the range of 1760-1770 cm^{-1} in the IR spectra of these compounds.



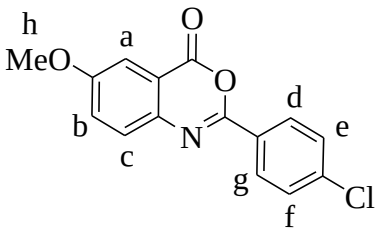
(165-167) **(168-175)**

Scheme: 4.5

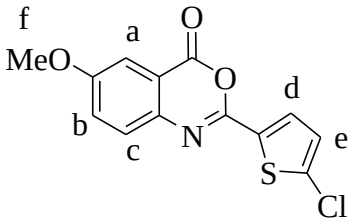
Table 4.2: Infra-red data of intermediate compounds **(168-175)**

Compound	X	Ar-Y	IR bands (cm^{-1})
168	6-OMe	4-Chlorophenyl	1759 (C=O stretch), 1625, 1497, 1030.
169	6,7-DiOMe	4-Chlorophenyl	1759 (C=O stretch), 1602, 1505, 1016.
170	6-Chloro	4-Chlorophenyl	1751 (C=O stretch), 1612, 1467,

			1255.
171	6-OMe	5-Chlorothiophenyl	1761 (C=O stretch), 1612, 1489, 1040.
172	6,7-DiOMe	5-Chlorothiophenyl	1760 (C=O stretch), 1602, 1506, 1015.
173	6-Chloro	5-Chlorothiophenyl	1767 (C=O stretch), 1615, 1430, 1028.
174	6,7-DiOMe	6-Chloropyridin-3-yl	1749 (C=O stretch), 1602, 1508, 1015.
175	6-Chloro	6-Chloropyridin-3-yl	1765 (C=O stretch), 1623, 1474, 1132.



(168)

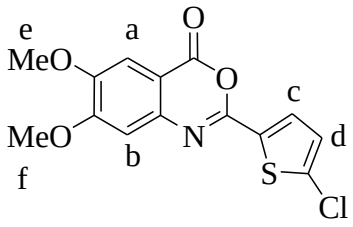


(171)

Compound (**168**) showed peak for C=O stretching at 1759 cm^{-1}. In its proton NMR spectrum signals are observed for aromatic protons at δ 7.65-7.64 (d, 1H*a*) and 7.55-7.53 (m, 4H*d-g*) as two separate multiplets for one and four protons respectively while another multiplet of one proton at δ 7.51-7.49 (d, *J* = 7.2 Hz, 1H*c*) and one doublet of doublet due to one proton appeared at δ 7.44-7.42 (dd, 1H*b*, *J* = 2.4 Hz and 7.2 Hz). A singlet of methoxy protons was seen at δ 3.96 (s, 3H*h*).

The IR spectrum of compound (**171**) revealed characteristic cyclic ester peak at 1761 cm^{-1} for C=O stretching. PMR spectrum of the compound exhibited two multiplets of aromatic protons at δ 7.71-7.70 (m, 1H*d*) and 7.62-7.61 (m, 1H*a*) for one proton each. One doublet by one proton at δ 7.58-7.56 (d, *J* = 7.2 Hz, 1H*c*) and one doublet of doublet due to one proton appeared at δ 7.41-7.39 (dd, 1H*b*, *J* = 2.2 Hz and 7.2 Hz). Another doublet was furnished by one thiophene

proton at δ 7.00 (d, *J* = 3.2 Hz, 1H*e*). A singlet at δ 3.94 (s, 3H*f*) was offered by one methoxy protons.



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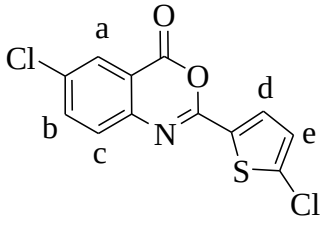
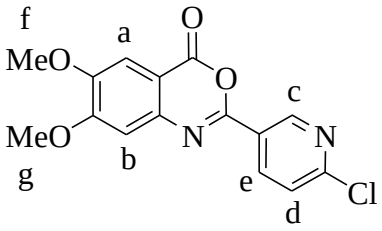


(173)

Compound (**172**) displayed C=O stretching of lactone at 1760 cm^{-1}. Proton NMR signals of the compound displayed presence of aromatic protons at δ 7.69-7.68 (d, *J* = 3.2 Hz, 1H*c*) as doublet and two individual singlets for one proton each at δ 7.52 (s, 1H*a*) and 7.03 (s, 1H*b*). Also a doublet due to one proton at 6.98-6.97 (d, *J* = 3.2 Hz, 1H*d*) and two singlets for six methoxy protons at δ 4.02 (s, 3H*e*) and 3.99 (s, 3H*f*) were observed.

Compound (**173**) containing lactone moiety showed C=O stretching at 1767 cm^{-1} in its IR spectrum. Proton NMR showed separate multiplets at δ 8.19-8.18 (m, 1H*a*) and 7.77-7.75 (m, 2H*b,d*) designating one and two protons respectively. A doublet due to *ortho* coupling at δ 7.59-7.57 (d, *J* = 6.8 Hz, 1H*c*) and another doublet for a single proton appeared at δ 7.03-7.02 (d, *J* = 3.2 Hz, 1H*e*).



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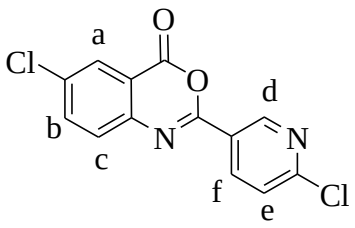


(175)

C=O stretching in cyclic ester was observed at 1749 cm^{-1} in the IR spectrum of compound (**174**). PMR spectrum of the compound revealed peaks at δ 9.27 (d, *J* = 2.0 Hz, 1H*c*) as doublet due to *meta* coupling with 1H*e* and δ 8.50-8.48 (dd, *J* = 2.0 and 6.8 Hz, 1H*e*) as doublet of doublet due to *ortho* and *meta* coupling in pyridine ring protons. Two separate singlets

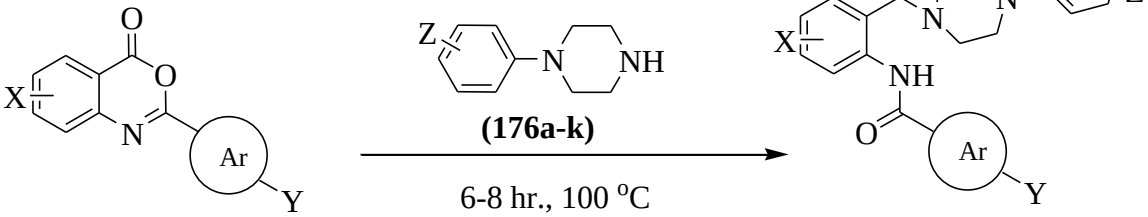
for two different protons at δ 7.59 (s, 1H*a*, Ar-*H*) and 7.14 (s, 1H*b*) and one doublet of single proton at δ 7.51-7.49 (d, *J* = 6.8 Hz, 1H*d*) were also revealed in aromatic region. Two different singlets representing two methoxy protons were displayed at δ 4.07 (s, 3H*f*) and 4.03 (s, 3H*g*).

A peak at 1765 cm^{-1} for C=O stretching in cyclic ester was revealed in compound (**175**). In its PMR spectrum a doublet at δ 9.29-9.28 (d, *J* = 2.0 Hz, 1H*d*) because of *meta* coupling and doublet of doublet at δ 8.52-8.50 (dd, *J* = 2.0 and 6.8 Hz, 1H*f*) due to *ortho* and *meta* coupling in two protons of pyridine ring were observed. A doublet at δ 8.25-8.24 (d, *J* = 2.0 Hz, 1H*a*) signifying one proton and a doublet of doublet at δ 7.84-7.82 (dd, *J* = 2.0 and 6.8 Hz, 1H*b*) due to coupling in phenyl ring protons were also observed. Two separate doublets of one proton each at δ 7.70-7.68 (d, *J* = 6.8 Hz, 1H*e*) and 7.53 (d, *J* = 6.8 Hz, 1H*c*) were also observed in aromatic region.

4.1.5: Synthesis of substituted anthranilamides (177-235)

The target compounds, diamide (anthranilamide) analogs were synthesized by reacting benz[*d*][1,3]oxazin-4-one derivatives with piperazine free base or piperazine hydrochloride in dry dimethylformamide and *N*,*N*-diisopropylethylamine after heating at 100 ^{0}C for 6-8 hrs (**scheme 4.6**). The IR spectra of these amide derivatives exhibited peak of C=O (amide carbonyl) stretching in the range of 1640-1680 cm^{-1}. 4-Nitrodiamide derivatives were further reduced to amino analogs using ferric powder and ammonium chloride in methanol.

Compound (**177**) in its IR spectrum displayed peaks at 1664 cm^{-1} for C=O stretching. Its PMR spectrum showed broad singlet at δ 9.66 (s, 1H*d*, N*H*CO) for amidic proton while aromatic proton signals appeared as three separate doublets, one doublet at δ 8.21-8.19 (d, *J* = 9.0 Hz, $1H_c$) representing one proton and two doublets due to *ortho* coupling among four protons at δ 7.87-7.85 (d, *J* = 8.5 Hz, $2H_{e,\ h}$) and 7.46-7.44 (d, *J* = 8.5 Hz, $2H_{f,\ g}$). A doublet of doublet at δ 7.37-7.35 (dd, *J* = 1.4, 7.8 Hz, $1H_m$) and two multiplets at δ 7.21-7.17 (m, $1H_b$) and 7.00-6.97 (m, $2H_{n,\ p}$) were also observed. Both *ortho* and *meta* coupling for H*o* proton furnished doublet of doublet at δ 6.93-6.91 (dd, *J* = 1.4, 7.8 Hz, $1H_o$) and separate doublet because of *meta* coupling of H_a with H_b proton was noted at δ 6.84-6.83 (d, *J* = 2.8 Hz, $1H_a$). Among the aliphatic protons,

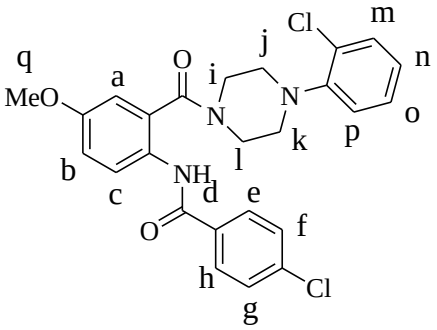


(168-175) **(177-235)**

Compound	Z	Compound	Z
176a	2-Cl	**176g**	4-Me
176b	2-F	**176h**	2-Me
176c	4-Cl	**176i**	4-NO_2
176d	4-F	**176j**	4-CN
176e	4-OMe	**176k**	2-CN
176f	2-OMe		

Scheme: 4.6

methylene protons from piperazine ring were observed as three broad singlets at δ 3.95 (bs, 2H*j*), 3.73 (bs, 2H*k*) and 3.02 (bs, 4H*i, l*). A singlet corresponding to methoxy protons also appeared at δ 3.82 (s, 3H*q*). The mass spectrum of compound (**177**) exhibited peak at m/z 484.2 $[M+H]^+$.



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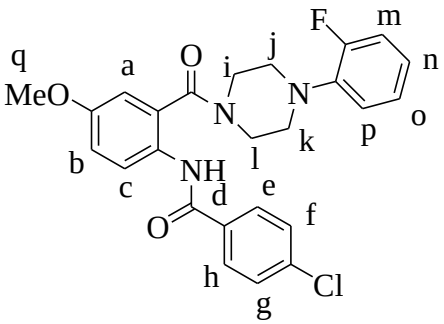
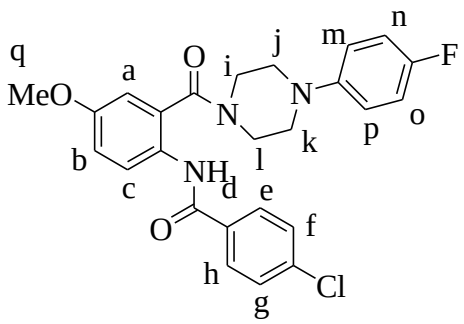


(178)

The IR spectrum of compound (**178**) showed peaks at 1664 cm^{-1} for C=O stretching. The PMR spectrum of the compound revealed a broad singlet for amide proton at δ 10.08 and a singlet for one proton at δ 8.09 (s, 1H$_c$). Two doublets for each of the two protons at δ 7.96-7.94 (d, *J* = 8.3 Hz, 2H$_{e, h,}$) and 7.49-7.47 (d, *J* = 8.4 Hz, 2H$_{f, g}$) along with three multiplets at δ 7.07-6.99 (m, 3H$_{m, n, o}$), 6.97-6.94 (m, 1H$_p$) and 6.89-6.86 (m, 2H$_{b, a}$) appeared for the aromatic protons. Signal for protons of the methoxy group was observed at δ 3.82 (s, 3H*q*) as a singlet and methylene protons of the piperazine ring were seen as individual broad singlets at δ 3.77 (bs, 2H*j*, C*H*$_2$), 3.54 (bs, 2H*k*) and 3.0 (bs, 4H*i, l*). Mass spectrum of the compound displayed $[M+1]^+$ peak at 468.4 m/z.

Compound (**179**) displayed signals for NH stretching at 3318 cm^{-1} and characteristic amide C=O stretching at 1678 cm^{-1} in its IR spectrum. The PMR spectrum of the compound revealed peaks at δ 9.66 (s, 1H$_d$) as broad singlet and doublets at δ 8.28-8.26 (d, *J* = 9.0 Hz, 1H$_c$) accounting for one proton and δ 7.87-7.86 (d, *J* = 8.5 Hz, 2H$_{e, h}$) with δ 7.47-7.46 (d, *J* = 8.5 Hz, 2H$_{f, g}$) both representing four aromatic protons. A doublet of doublet for one proton at 7.06-7.04 (dd, *J* = 3.0, 9.0 Hz, 1H$_b$) and multiplets for two and three aromatic protons also appeared at δ 7.00-6.97 (m, 2H*n, o*) and 6.89-6.85 (m, 3H*m, p, a*) respectively. The piperazine methylene protons merged with methoxy protons as a multiplet at δ 3.85 (m, 7H*j, k, q*) while other four methylene protons as broad singlet were seen at δ 3.11 (bs, 4H*i, l*).



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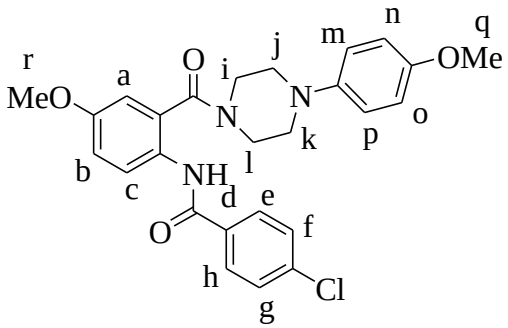
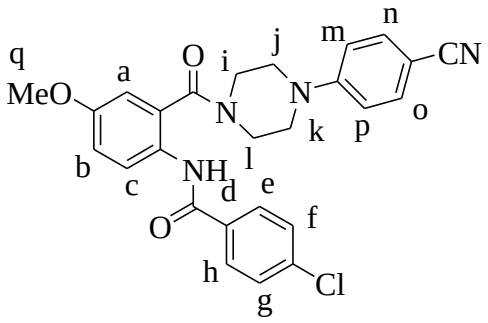


(180)

Compound (**180**) offered peaks at 3313 cm^{-1} for NH stretching and 1666 cm^{-1} for C=O stretching in its IR spectrum. Its proton NMR revealed an amide proton singlet at δ 9.63 (s, 1H*d*, N*H*CO) while the aromatic region displayed signals of three doublets at δ 8.28-8.26 (d, *J* = 9.0

Hz, 1H*c*), 7.87-7.85 (d, *J* = 8.5 Hz, 2H*e, h*) and 7.47-7.45 (d, *J* = 8.5 Hz, 2H*f, g*), all accounting *ortho* coupling among them. Multiplets for four protons and one proton appeared at δ 7.06-7.02 (m, 4H*m, n, o, p*) and 6.89-6.86 (m, 1H*b*) respectively, followed by an upfield doublet for a single proton at δ 6.85-6.84 (d, *J* = 3.0 Hz, 1H*a*). A broad singlet for two methylene protons of piperazine was observed at δ 3.93 (bs, 2H*j*, CH_2) along with six methoxy protons as a multiplet at δ 3.83 (m, 6H*q, r*) and two singlets at δ 3.74 (bs, 2H*k*) and 3.06 (s, 4H*i, l*) in the aliphatic region were also noticed.

Signals for NH stretching at 3241 cm^{-1} with a noticeable CN stretching at 2209 cm^{-1} and amide C=O band at 1640 cm^{-1} were observed in the IR spectrum of compound (**181**). PMR spectrum of the compound exhibited a broad singlet for amide at δ 9.58 (s, 1H, NH_d) and a doublet for a proton at δ 8.23-8.21 (d, *J* = 9.0 Hz, 1H*c*). Three doublets each corresponding to two protons were noticed at δ 7.84-7.82 (d, *J* = 8.5 Hz, 2H*e, h*), 7.52-7.50 (d, *J* = 8.5 Hz, 2H*f, g*) and 7.45-7.43 (d, *J* = 8.5 Hz, 2H*n, o*). A doublet of doublet at δ 7.05-7.04 (dd, *J* = 3.0, 9.0 Hz, 1H*b*) and a multiplet for three protons at δ 6.85-6.82 (m, 3H*m, p, a*) were also observed. Four methylene protons of piperazine ring merged with three protons of methoxy group appeared as multiplet at δ 3.83-3.82 (m, 7H*j, k, q*) whereas the other methylenes were seen at δ 3.35 (bs, 4H*i, l*). Mass spectrum of the compound displayed quasimolecular ion peak at *m/z* 475.4 $[M+H]^+$



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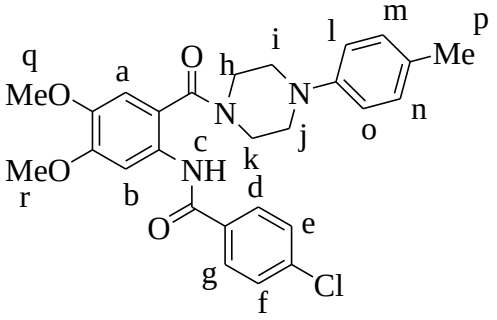
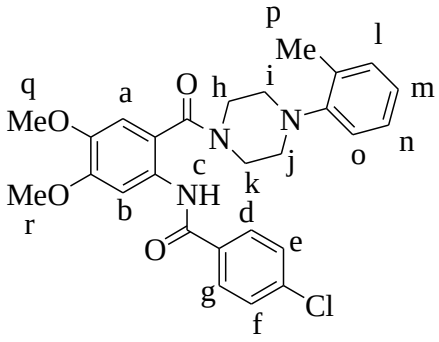


(182)

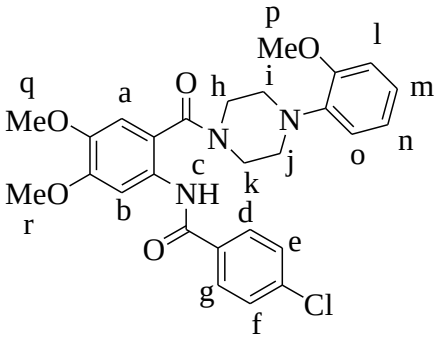
Compound (**182**) in its IR spectrum exhibited peak for C=O stretching at 1668 cm^{-1}. The PMR spectrum of the compound showed a singlet for amide proton at δ 10.37 (s, 1H*c*), a singlet at δ 8.19 (s, 1H*b*) for one proton and three doublets each representing two aromatic protons at δ 7.89-7.87 (d, *J* = 8.5 Hz, 2H*d, g*), δ 7.47-7.45 (d, *J* = 8.5 Hz, 2H*e, f*) and δ 7.09-7.07 (d, *J* = 8.3

Hz, 2H*l, o*). A multiplet for three aromatic protons appeared at δ 6.84-6.80 (m, 3H*a, m, n*). Signals were seen at δ 3.98 (s, 3H*r*) and 3.87 (s, 3H*q*) as two singlets for methoxy protons, at δ 3.82 (bs, 4H*i, j*) and 3.16 (bs, 4H*h, k*) as two broad singlets for piperazine methylene protons and at δ 2.27 (s, 3H*p*) as a singlet for methyl protons.

Compound (**183**) in its IR spectrum displayed peaks at 3318 cm^{-1} for NH stretching and 1661 cm^{-1} for C=O stretching. The PMR spectrum of the compound offered a singlet for amide proton at δ 10.32 (s, 1H*c*) and a singlet at δ 8.17 (s, 1H*b*) for single aromatic proton. Other aromatic protons accounting two doublets appeared at δ 7.91-7.90 (d, *J* = 8.8 Hz, 2H*d, g*) and 7.49-7.48 (d, *J* = 8.8 Hz, 2H*e, f*). Two multiplets for three protons at δ 7.19-7.16 (m, 2H*m, n*) and 7.01 (m, 1Ho) along with a doublet of doublet for one proton at δ 6.94-6.92 (dd, *J* = 1.2, 8.0 Hz, 1H*l*) and a singlet at δ 6.82 (s, 1H*a*) were also observed. Singlets for six methoxy protons at δ 3.97 (s, 3H*b*, OCH_3) and 3.87 (s, 3Ha, OCH_3), broad singlets of piperazinyl methylene protons at δ 3.81 (bs, 4H*i, j*) and 2.91 (bs,4H*h, k*), and a singlet for methyl protons at δ 2.31 (s, 3H*p*, CH_3) were seen in the aliphatic region. Carbon NMR of compound (**183**) revealed signals at δ 169.96, 164.12, 151.15, 150.51, 144.57, 138.26, 132.83, 132.78, 132.69, 131.21, 129.09, 128.61, 126.69, 123.97, 119.19, 114.65, 111.02, 106.23 for keto and aromatic and δ 56.40, 56.12, 52.04, 17.74 for aliphatic carbons. The HR-MS spectrum of the compound offered peak at *m/z* 494.1826 $[M+H]^+$.



(183)

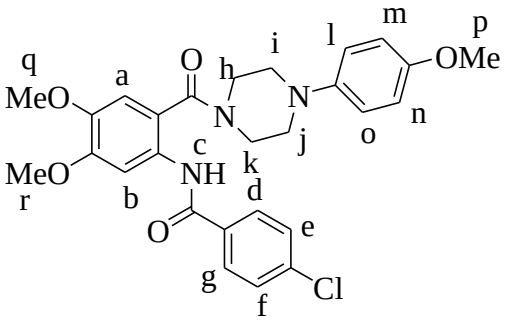


(184)

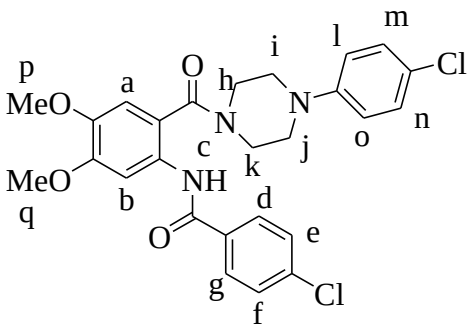
Compound (**184)** showed peaks at 3307 cm^{-1} for NH stretching and 1667 cm^{-1} for C=O stretching in its IR spectrum. PMR spectra of compound (**184**) displayed a singlet at δ 10.39 (s, 1H*c*) for -NH proton and another singlet at δ 8.22 (s, 1H*b*) for one aromatic proton. Two

doublets corresponding to four aromatic protons at δ 7.92-7.91 (d, *J* = 8.5 Hz, 2H*d, g*) and 7.50-7.49 (d, *J* = 8.5 Hz, 2H*e, f*), two multiplets accounting for four protons at δ 7.08-7.04 (m, 1H*o*) and 6.95-6.90 (m, 3H*l, m, n*) and a singlet for one proton at δ 6.84 (s, 1H*a*) appeared in aromatic region. Aliphatic protons revealed peaks at δ 4.00 (s, 3H*r*) as singlet for methoxy protons and δ 3.91-3.86 (m, 10H*a, q, i. j*) as a multiplet for six methoxy and four methylene protons together. A singlet due to four methylene protons was also seen at δ 3.10 (s, 4H*h, k*).

Compound (**185**) in its IR spectrum offered peaks at 3313 cm^{-1} for NH stretching and 1666 cm^{-1} for C=O stretching. In its ^{1}H-NMR spectrum the compound showed a singlet at δ 10.34 (s, 1Hc) for amide proton and another at δ 8.18 (s, 1H*b*) for an aromatic proton. Two doublets for four protons at δ 7.88-7.86 (d, *J* = 8.4 Hz, 2H*d, g*) and 7.47-7.45 (d, *J* = 8.4 Hz, 2H*e, f*) and a multiplet expressing five protons at δ 6.89-6.80 (m, 5H*a, l, m, n, o*) appeared in the aromatic region. Two singlets for six protons of methoxy group at δ 3.97 (s, 3H*r*) and 3.87 (s, 3H*q*) with three singlets at δ 3.82 (bs, 4H*i, j*), 3.75 (s, 3H*p*), 3.07 (bs, 4H*h, k*) were observed for aliphatic protons. ^{13}C-NMR revealed signals at δ 169.93, 164.07, 154.55, 151.23, 144.94, 144.52, 138.23, 132.92, 132.81, 129.07, 128.58, 118.91, 114.55, 114.34, 111.03, 106.18 and aliphatic carbon signals at δ 56.41, 56.12, 55.52, and 51.24 for aliphatic carbons. The HR-MS of the compound yielded a peak at *m/z* 510.1790 $[M+H]^{+}$



(185)

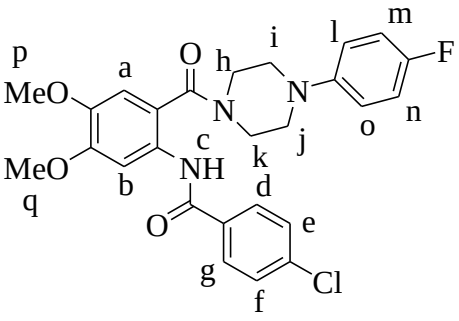


(186)

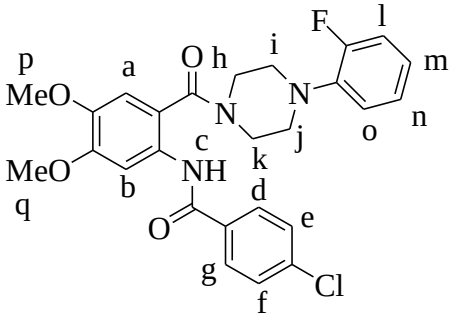
The IR spectrum of compound (**186**) revealed peaks for NH stretching at 3318 cm^{-1} and for C=O stretching at 1667 cm^{-1}. The PMR spectrum of the compound displayed two singlets at δ 10.31 (s, 1H*c*) and 8.18 (s, 1H*b*) for amide and an aromatic proton respectively. Peaks at δ 7.88-7.86 (d, *J* = 8.4 Hz, 2H*d, g*), 7.48-7.46 (d, *J* = 8.4 Hz, 2H*e, f*), 7.24-7.22 (d, *J* = 8.8 Hz,

2H*m, n*) and 6.88-6.86 (d, *J* = 8.4 Hz, 2H*l, o*) appeared as four individual doublets for the aromatic protons in addition to a singlet for aromatic proton at δ 6.80 (s, 1H*a*). Aliphatic protons appeared at δ 3.98 (s, 3H*q*) and 3.87 (s, 3H*p*) as two singlets for six protons of methoxy group and at δ 3.85 (bs, 4H*i. j*) and 3.18 (bs, 4H*h. k*) as two broad singlets for methylene protons. ^{13}C - NMR revealed peaks at δ 164.09, 151.39, 149.29, 138.30, 133.11, 132.79, 129.16, 129.10, 128.58, 117.87, 111.05, and 106.33 and at δ 56.47, 56.15, and 49.73 for aliphatic carbons. The mass spectrum of compound displayed $[M+H]^+$ peak at *m/z* 514.5

In the IR spectrum of compound (**187**) peak for C=O stretching was revealed at 1666 cm^{-1}. The PMR spectrum of the compound displayed two singlets at δ 10.35 (s, 1Hc, N*H*CO) for amide proton and 8.18 (s, 1H*b*) for an aromatic proton. Two doublets each representing two aromatic protons at δ 7.89-7.87 (d, *J* = 8.5 Hz, 2H*d, g*) and 7.48-7.46 (d, *J* = 8.5 Hz, 2H*e, f*) also appeared along with two multiplets corresponding to four protons at δ 6.99-6.95 (m, 2H*m, n*) and 6.88-6.85 (m, 2H*l, o*). A singlet for aromatic proton was also observed at δ 6.80 (s, 1H*a*). Six protons for methoxy group appeared at δ 3.98 (s, 3H*q*) and 3.88 (s, 3H*p*) as two singlets while eight piperazine methylene protons were noticed at δ 3.83 (bs, 4H*i, j*) and 3.11 (bs, 4H*h, k*) as two broad singlets. The HR-MS offered a mass peak at *m/z* 498.1590 $[M+H]^+$



(187)

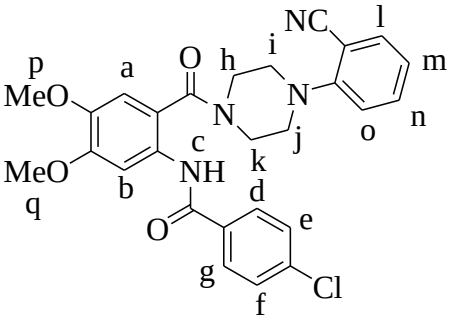


(188)

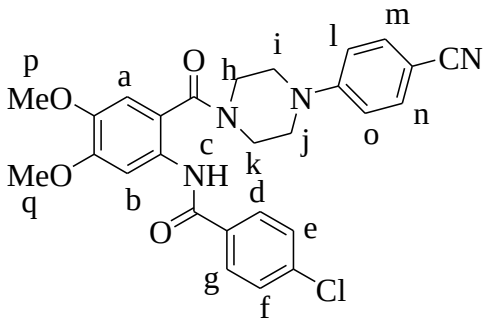
Compound (**188**) in its IR spectrum exhibited peaks at 3314 cm^{-1} for N-H stretching and 1683 cm^{-1} for C=O stretching. Its PMR spectrum showed signals at δ 10.40 (s, 1H*c*) and 8.21 (s, 1H*b*) as two different singlets, two doublets accounting for four aromatic protons at δ 7.92-7.91 (d, *J* = 8.5 Hz, 2H*d, g*) and 7.50-7.49 (d, *J* = 8.5 Hz, 2H*e, f*) and three separate multiplets for four different protons at δ 7.09-7.06 (m, 2Hl, n), 7.04-6.97 (m, 1H*m*) and 6.94-6.90 (m, 1H*o*). There

also appeared a singlet peak at δ 6.83 (s, 1H*a*) for one aromatic proton. Aliphatic protons were seen at δ 4.00 (s, 3H*q*) and 3.90 (s, 3H*p*) as two singlets of methoxy protons with δ 3.88 (bs, 4H*i, j*) and 3.12 (bs, 4H*h, k*) as broad singlets. ^{13}C-NMR spectrum revealed signals for carbons at δ 169.91, 164.08, 154.73, 151.18, 144.52, 139.27, 139.21, 138.24, 132.87, 132.78, 129.07, 128.59, 124.55, 123.38, 123.32, 119.20, 116.37, 116.21, 110.92, 106.16, 56.37, 56.12 and 50.78.

Compound (**189**) in its IR spectrum displayed peaks at 3304 cm^{-1} for N-H stretching along with the characteristic CN stretching at 2220 cm^{-1} and 1670 cm^{-1} for C=O stretching. The PMR spectrum of the compound revealed a singlet at δ 10.41 (s, 1H*c*) for amide proton and another singlet at δ 8.18 (s, 1H*b*) for one aromatic proton, a doublet comprising two protons at δ 7.92-7.90 (d, *J* = 8.5 Hz, 2H*d, g*) and three individual multiplets representing one proton each at δ 7.62-7.60 (m, 1H*e*), 7.53-7.48 (m, 3H*f, l, n*) and 7.09 (m, 1H*m*). A doublet for a proton at δ 6.99-6.98 (d, *J* = 8.5 Hz, 1H*o*) and a singlet at δ 6.82 (s, 1H*a*) in aromatic region were also observed. The methoxy protons appeared as two singlets at δ 4.00 (s, 3Hq) and 3.91 (s, 3H*p*) while piperazine methylene protons were seen at δ 3.94 (s, 4H*i, j*) and 3.21 (bs, 4H*h, k*) as broad singlets.



(189)

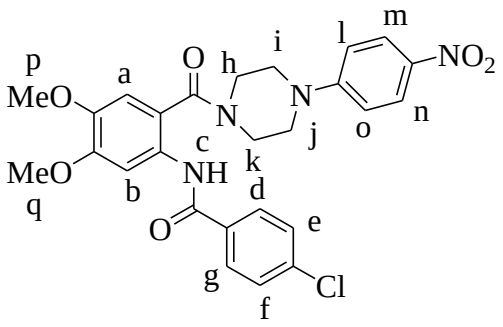


(190)

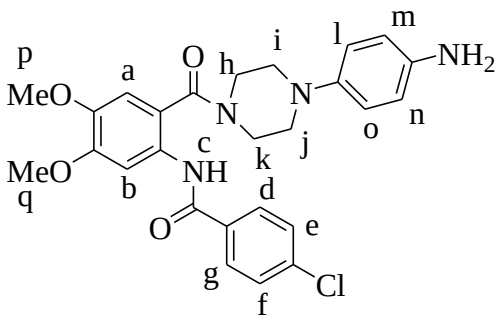
Compound (**190**) showed signals for N-H stretching at 3312 cm^{-1}, for CN stretching at 2210 cm^{-1} and C=O stretching at 1683 cm^{-1} in its IR spectra. ^{1}H-NMR spectrum of the compound offered peaks at δ 10.39 (s, 1H*c*, -N*H*CO) as singlet for amide proton and δ 8.21 (s, 1H*b*) singlet for one aromatic proton. Other aromatic protons appeared as four doublets each corresponding to two protons at δ 7.90-7.89 (d, *J* = 9.0 Hz, 2H*d, g*), 7.55-7.53 (d, *J* = 9.0 Hz, 2H*e, f*), 7.50-7.48 (d, *J* = 9.0 Hz, 2H*m, n*) and 6.89-6.87 (d, *J* = 9.0 Hz, 2H*l, o*) along with one

proton singlet at δ 6.82 (s, 1H*a*, Ar-*H*). Aliphatic protons were observed at δ 4.01 (s, 3H*q*) and 3.90 (s, 3H*p*) as two singlets for six protons for methoxy group and at δ 3.86 (bs, 4H*i*, *j*) and 3.40 (bs, 4H*h*, *k*) as two broad singlets for piperazinyl methylene protons.

Compound (**191**) furnished peaks at 1668 cm^{-1} for C=O stretching along with 1521 and 1325 cm^{-1} for N-O stretching in its IR spectrum. Proton NMR of the compound exhibited singlets for two different protons at δ 10.40 (s, 1H*c*, -N*H*CO) and δ 8.20 (s, 1H*b*) as well as three separate doublets each for two protons at δ 8.17-8.15 (d, *J* = 9.0 Hz, 2H*m*, *n*), 7.91-7.89 (d, *J* = 8.5 Hz, 2H*d*, *g*) and δ 7.50-7.48 (d, *J* = 8.5 Hz, 2H*e*, *f*), and a multiplet for three aromatic protons at δ 6.85-6.83 (m, 3H*a*, *l*, *o*). Aliphatic protons appeared at δ 4.01 (s, 3H*q*) and 3.90 (s, 3H*p*) as two singlets for six hydrogens of methoxy group in addition to δ 3.89 (bs, 4H*i*, *j*) and 3.51 (bs, 4H*h*, *k*) as broad singlets for eight piperazinyl protons.



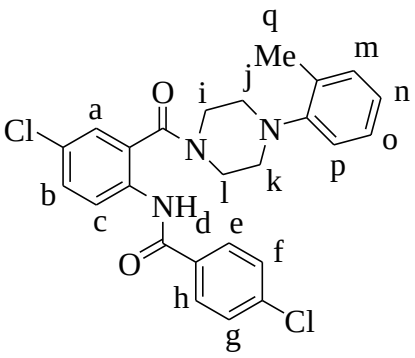
(191)



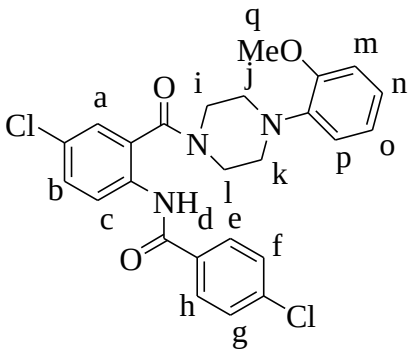
(192)

Peaks were observed at 3350 cm^{-1} for N-H stretching and 1667 cm^{-1} for C=O stretching in the IR spectrum of compound (**192**). The ^{1}H-NMR spectrum showed peaks at δ 10.38 (s, 1Hc, -N*H*CO) and 8.21 (s, 1Hb) as two singlets for two protons and δ 7.91-7.89 (d, *J* = 8.5 Hz, 2H*d*, *g*), 7.49-7.48 (d, *J* = 8.5 Hz, 2H*e*, *f*) as two doublets representing four aromatic protons. A multiplet for three protons at δ 6.83-6.79 (m, 3H*a*, *l*, *o*) and a doublet for two protons at δ 6.67-6.65 (d, *J* = 8.5 Hz, 2H*m*, *n*) were also noticed. Two isolated singlets for methoxy hydrogens at δ 4.00 (s, 3Hq) and 3.89 (s, 3Hp) and two broad singlets at δ 3.83 (bs, 4H*i*, *j*) and 3.06 (bs, 4H*h*, *k*) for methylene protons too were displayed in aliphatic region.

Compound (**193**) in the IR spectrum displayed signal for C=O stretching at 1673 cm^{-1} while its PMR spectrum revealed a singlet for amide proton at δ 10.03 (s, 1H*d*, N*H*CO). Two doublets both due to *ortho* coupling were displayed at δ 8.44-8.42 (d, *J* = 8.8 Hz, 1H*c*) and 7.88-7.86 (d, *J* = 8.4 Hz, 2H*e*, *h*), while a doublet signifying *meta* coupling for one proton at δ 7.30 (d, *J* = 2.4 Hz, 1H*a*) and four different multiplets corresponding to aromatic protons were observed at δ 7.49-7.43 (m, 3H*f*, *g*, *b*), 7.20-7.13 (m, 2H*m*, *o*), 7.01 (m, 1H*n*) and 6.95-6.93 (m, 1H*p*). Two broad singlets of piperazinyl methylene protons at δ 3.81 (bs, 4H*j*, *k*) and 2.92 (bs, 4H*i*, *l*) and a singlet for methyl protons at δ 2.31 (s, 3H*q*, CH_3) were seen in aliphatic region. ^{13}C-NMR of compound gave peaks at δ 168.21, 164.11, 150.40, 138.54, 136.15, 132.71, 132.48, 131.24, 131.11, 129.15, 128.81, 128.69, 127.56, 126.73, 124.37, 124.20, 124.07, 119.25, 52.00 and 17.74.



(193)

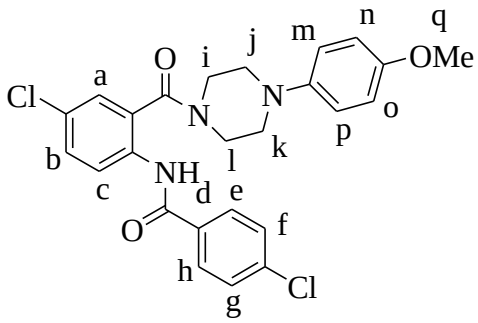


(194)

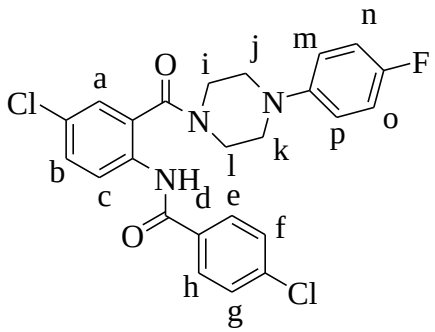
Compound (**194**) showed peaks at 3239 cm^{-1} for NH stretching and 1674 cm^{-1} for C=O stretching in its IR spectrum. The PMR spectrum of the compound displayed a singlet for amidic proton at δ 10.08 (s, 1H*d*, N*H*CO) and two doublets at δ 8.46-8.44 (d, *J* = 8.5 Hz, 1H*c*) and 7.90-7.88 (d, *J* = 8.5 Hz, 2H*e*, *h*) for one proton and two protons respectively. Other aromatic protons provided a multiplet at δ 7.50-7.45 (m, 3H*f*, *g*, *b*), a doublet at δ 7.32 (d, *J* = 2.5 Hz, 1H*a*) and two multiplets at δ 7.08-7.04 (m, 1H*m*) and 6.94-6.88 (m, 3H*n*, *o*, *p*). A singlet for methoxy protons at δ 3.89 (s, 3Hq) and three broad singlets for methylene protons were also noticed at δ 3.97 (bs, 2H*j*), 3.77 (bs, 2H*k*) and 3.10 (bs, 4H*i*, *l*).

In its IR spectrum compound (**195**) exhibited peaks at 3269 cm^{-1} for NH stretching and 1683 cm^{-1} for C=O stretching. The compound in its proton NMR spectrum offered peaks at δ

10.02 (s, 1H*d*, N*H*CO) as a singlet and a doublet at δ 8.44-8.42 (d, *J* = 8.8 Hz, 1H*c*). A doublet for two protons δ 7.86-7.84 (d, *J* = 8.8 Hz, 2H*e, h*), a multiplet for three protons at δ 7.47-7.43 (m, 3H*f, g, b*) and a doublet due to *meta* coupling for a proton at δ 7.29-7.28 (d, *J* = 2.4 Hz, 1H*a*) along with a multiplet representing four protons appeared at δ 6.89-6.82 (m, 4H*m, n, o, p*). Aliphatic protons offered a multiplet corresponding to seven protons at δ 3.80-3.76 (m, 7H*j, k, q*) and a singlet for methylene protons at δ 3.07 (s, 4H*i, l*). The HR-MS of the compound furnished $[M+H]^+$ peak at *m/z* 484.1189.



(195)

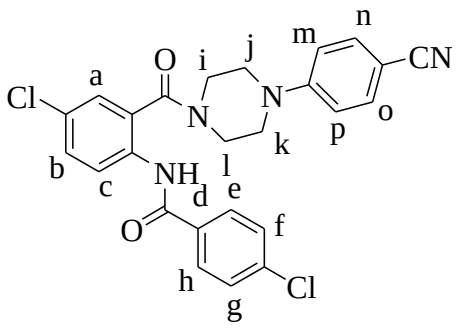


(196)

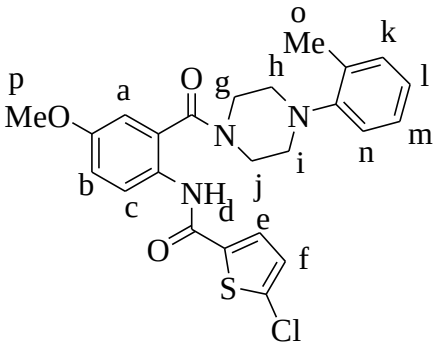
Compound (**196**) revealed peaks for NH and C=O stretching at 3317 and 1681 cm^{-1} respectively whereas its PMR spectrum showed a singlet for the amide proton at δ 10.03 (s, 1H*d*, N*H*CO). Two doublets accounting three protons were seen at δ 8.46-8.44 (d, *J* = 9.0 Hz, 1H*c*) and 7.87-7.85 (d, *J* = 9.0 Hz, 2H*e, h*) while other aromatic protons gave multiplets at δ 7.48-7.45 (m, 3H*f, g, b*) for three protons, δ 7.29 (d, *J* = 2.5 Hz, 1H*a*) for a proton and δ 6.99-6.96 (m, 2H*n, o*) and 6.88-6.86 (m, 2H*m, p*) for two protons each. Aliphatic protons were displayed at δ 3.82 (bs, 4H*j, k*) and 3.12 (s, 4H*i, l*) as two broad singlets. ^{13}C-NMR of the compound revealed signals at δ 168.21, 164.11, 138.57, 136.23, 132.42, 131.27, 129.14, 128.66, 128.55, 127.53, 124.55, 124.28, 118.81, 118.73, 115.90, 115.68 and 50.82. Mass spectrum of the compound showed $[M+H]^+$ peak at *m/z* 472.4.

Signals were seen in the IR spectrum of compound (**197**) at 3212 cm^{-1} for NH stretching with a distinguished peak at 2211 cm^{-1} for CN stretching and 1639 cm^{-1} for C=O stretching. The proton NMR spectrum of the compound showed a singlet at δ 10.02 (s, 1H*d*, N*H*CO) and a multiplet at δ 8.38-8.34 (m, 1H*c*). Aromatic region revealed two doublets for two protons each at δ 7.84-7.82 (d, *J* = 8.4 Hz, 2H*e, h*) and 7.52-7.50 (d, *J* = 8.8 Hz, 2H*n, o*) and a multiplet for three

protons at δ 7.46-7.43 (m, 3H*f, g, b*) as well as two doublets at δ 7.28-7.27 (d, *J* = 2.4 Hz, 1H*a*) and 6.86-6.85 (d, *J* = 8.8 Hz, 2H*m, p*). Aliphatic protons from piperazine ring showed two singlets at δ 3.83 (bs, 4H*j, k*), 3.37 (s, 4H*i, l*). In its ^{13}C-NMR spectrum signals were displayed at δ 168.50, 164.17, 152.73, 138.61, 136.21, 133.66, 132.30, 131.45, 129.14, 128.75, 128.69, 127.49, 124.61, 119.59, 114.76, 101.75 and 48.70.



(197)

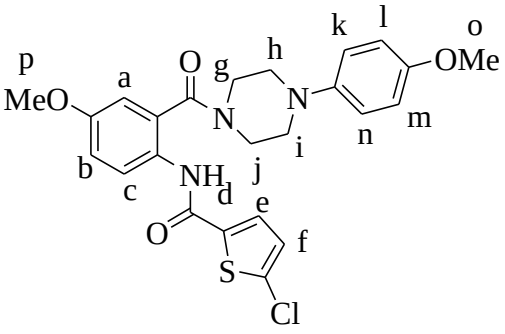


(198)

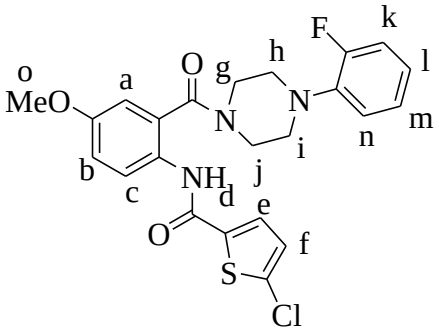
The IR spectrum of compound (**198**) showed peaks at 3291 cm^{-1} for NH stretching and 1652 cm^{-1} for C=O stretching. Its proton NMR displayed signals at δ 9.60 (s, 1H*d*, N*H*CO) singlet for amide NH proton, two doublets at δ 8.20-8.18 (d, *J* = 9.0 Hz, 1H*c*) and 7.43 (d, *J* = 3.5 Hz, 1H*e*) and multiplets for two protons each at δ 7.22-7.16 (m, 2H*k, m*), 7.05-7.00 (m, 2H*l, n*) and 6.97-6.95 (m, 2H*a, b*). A doublet for a single proton appeared at δ 6.86 (d, *J* = 3.5 Hz, 1H*f*). Aliphatic region displayed methoxy protons at δ 3.84 (s, 3H*p*, OC*H*$_3$), methylene protons at δ 3.74 (bs, 4H*h, i*) and 2.93 (bs, 4H*g, j*) while the methyl protons appeared at δ 2.34 (s, 3H*o*). ^{13}C-NMR spectrum exhibited carbon signals at δ 169.98, 158.87, 155.53, 150.55, 138.24, 136.11, 132.73, 131.23, 129.62, 127.55, 127.25, 126.74, 125.67, 124.79, 123.99, 119.27, 115.79, 113.49, 55.69 and 17.79.

Compound (**199**) furnished peaks for NH stretching at 3267 cm^{-1} and C=O stretching of amide at 1658 cm^{-1} in its IR spectrum. PMR spectrum of the compound offered singlet of amide -NH proton at δ 9.59 (s, 1H*d*, N*H*CO) followed by individual doublets at δ 8.19-8.18 (d, *J* = 9.0 Hz, 1H*c*) and 7.41 (d, *J* = 4.0 Hz, 1H*e*), a doublet of doublet at δ 7.03-7.01 (dd, *J* = 3.0 & 9.0 Hz, 1H*b*) and another doublet at δ 6.95-6.94 (d, *J* = 4.0 Hz, 1H*f*) each representing a single proton. A multiplet for five aromatic protons appeared at 6.91-6.84 (m, 5H*a, k, l, m, n*). Broad singlets due

to piperazinyl protons were observed at δ 3.95 (bs, 4H*h, i*) and 3.08 (bs, 4H*g, j*) while the methoxy protons were displayed at δ 3.84 (s, 3H*o*) and 3.79 (s, 3H*p*) as singlets.



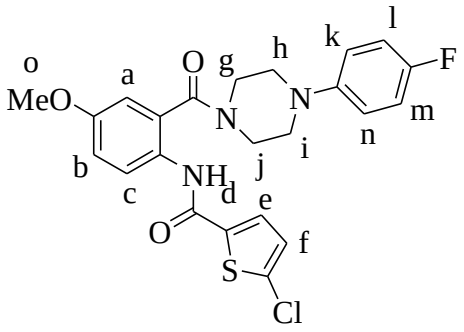
(**199)**



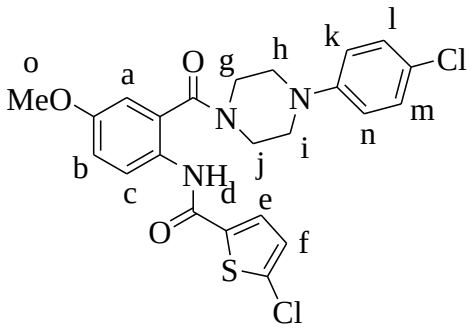
(200)

Compound (**200**) in its IR spectrum exhibited peaks at 3313 cm^{-1} for NH stretching and 1666 cm^{-1} for C=O stretching. ^{1}H-NMR spectrum of the compound offered singlet for amide proton at δ 9.65 (s, 1H*d*, N*H*CO) and doublets each accounting two protons at 8.30-8.29 (d, *J* = 9.0 Hz, 1H*c*), 7.89-7.87 (d, *J* = 8.5 Hz, 2H*k, m*) and 7.49-7.47 (d, *J* = 8.5 Hz, 2H*l, n*). Other aromatic protons appeared at δ 7.07-7.04 (m, 2H*b, e*), 6.92-6.88 (m, 1H*a*) and 6.87-6.86 (d, *J* = 3.0 Hz, 1H*f*). Methylene protons of piperazine merged with three methoxy hydrogens appeared as multiplet at δ 3.96-3.77 (m, 7H*h, i, p*) and as a singlet at δ 3.09 (s, 4H*g, j*).

The IR spectrum of compound (**201**) revealed peaks for NH stretching at 3306 cm^{-1} and C=O stretching at 1651 cm^{-1}. Its PMR spectrum furnished singlet at δ 9.58 (s, 1H*d*, N*H*CO) for the amide proton and two doublets for four protons at δ 8.20-8.18 (d, *J* = 9.0 Hz, 1H*c*) and 7.41-7.40 (d, *J* = 3.5 Hz, 1H*e*) and a multiplet for three protons at δ 7.03-6.97 (m, 3H*b, l, m*). A doublet accounting one proton at δ 6.95-6.94 (d, *J* = 4.0 Hz, 1H*a*) and a multiplet accounting two protons at δ 6.90-6.87 (m, 2H*k, n*) and a doublet for one proton at δ 6.84 (d, *J* = 3.5 Hz, 1H*f*) appeared in aromatic region. A multiplet for methoxy and methylene protons were observed at δ 3.84 (m, 7H*h, i, o*) followed by broad singlet at δ 3.13 (bs, 4H*g, j*). The carbon NMR offered signals at δ 169.04, 159.00, 158.70, 156.79, 155.73, 147.40, 138.17, 136.06, 129.27, 127.69, 127.24, 126.11, 125.26, 118.78, 115.84, 115.80, 115.67, 113.39, 55.68 and 50.76. The HR-MS of the compound displayed $[M+H]^+$ peak at *m/z* 474.1049.



(201)

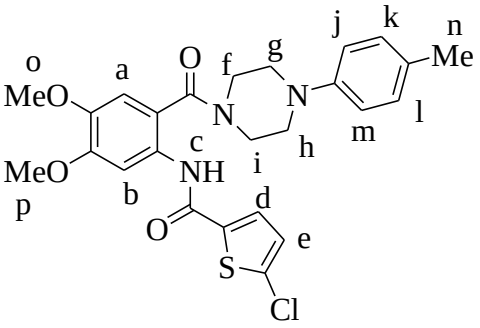


(202)

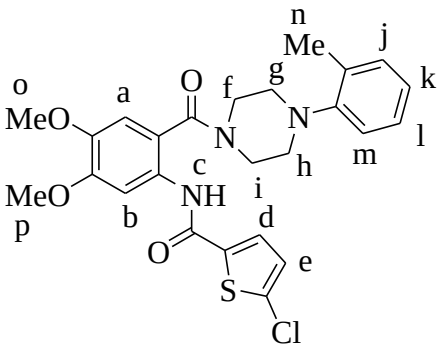
Compound (**202**) offered IR peaks at 3297 cm^{-1} for NH stretching and 1650 cm^{-1} for C=O stretching. ^{1}H-NMR showed peaks at δ 9.57 (s, 1H*d*, N*H*CO) as a singlet of amide proton and δ 8.20-8.18 (d, *J* = 9.0 Hz, 1H*c*) and δ 7.40-7.39 (d, *J* = 4.0 Hz, 1H*e*) as two individual doublets representing two protons along with a multiplet for two protons at δ 7.25-7.24 (m, 2H*l, m*). A doublet of doublet for H*b* proton coupled with H*a* and H*c* protons appeared at δ 7.04-7.02 (dd, *J* = 3.0 & 9.0 Hz, 1H*b*). Peaks at δ 6.95-6.94 (d, *J* = 4.0 Hz, 1H*f*) and 6.86-6.83 (m, 3H*a, k, n*) were offered by other aromatic protons. A multiplet at δ 3.84 (m, 7H*h, i, o*) and broad singlet at δ 3.18 (bs, 4H*g, j*) were observed for aliphatic protons. The HR-MS of the compound gave $[M+H]^{+}$ peak at *m/z* 490.0753.

Compound (**203**) showed peaks at 3282 cm^{-1} for N-H stretching and 1647 cm^{-1} for C=O stretching in its IR spectrum. In its ^{1}H-NMR spectrum the compound displayed peaks at δ 10.31 (s, 1H*c*) as singlet for amide proton and a singlet at δ 8.08 (s, 1H*b*) for one aromatic proton. Four different doublets were noticed at δ 7.43-7.42 (d, *J* = 4.0 Hz, 1H*d*) for one thiophene proton, δ 7.10-7.08 (d, *J* = 8.3 Hz, 2H*k, l*) for two aromatic protons along with δ 6.94-6.93 (d, *J* = 4.0 Hz, 1H*e*) for another thiophene proton and δ 6.85-6.82 (d, *J* = 8.4 Hz, 2H*j, m*) for two other aromatic protons. A singlet also appeared at δ 6.79 (s, 1H*a*) for an aromatic proton. In aliphatic region, two singlets for six protons of methoxy group at δ 3.95 (s, 3H*p*) and 3.86 (s, 3H*o*) and two broad singlets for eight piperazinyl protons were displayed at δ 3.82 (bs, 4H*g, h*) and δ 3.16 (bs, 4H*f, i*) with a singlet of methyl protons at δ 2.27 (s, 3H*n*). ^{13}C-NMR of the compound displayed signals at δ 170.04, 158.95, 151.34, 148.60, 144.52, 138.36, 136.32, 132.74, 130.49, 129.83, 127.63, 127.33, 117.07, 113.86, 111.12, 105.97 for amide and aromatic carbons and δ 56.43, 56.15,

50.35, 20.45 for aliphatic carbons. The HR-MS of compound offered $[M+H]^+$ peak at *m/z* 500.1405.



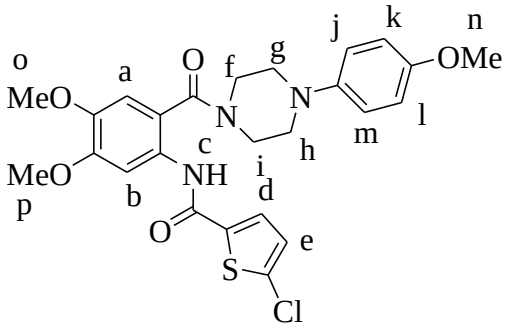
(203)



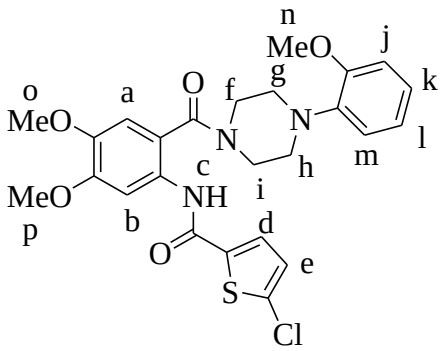
(204)

Compound (**204**) offered signals at 3281 cm^{-1} for N-H stretching and 1655 cm^{-1} for C=O stretching in its IR spectrum. PMR spectrum of the compound revealed two separate singlets at δ 10.34 (s, 1H*c*, N*H*CO) for amide proton and δ 8.13 (s, 1H*b*) for one aromatic proton. A doublet for one thienyl proton at δ 7.46 (d, *J* = 4.0 Hz, 1H*d*) and three individual multiplets at δ 7.23-7.17 (m, 2H*j, l*) for two aromatic protons, δ 7.06-7.03 (m, 1H*e*) for thiophene proton, δ 7.00-6.98 (m, 2H*k, m*) for two aromatic protons and a singlet for a proton at δ 6.84 (s, 1Ha) also appeared. Two singlets corresponding to six protons of methoxy group at δ 3.98 (s, 3Hp) and 3.90 (s, 3H*o*), two broad singlets for piperazinyl protons at δ 3.85 (bs, 4H*g, h*,) and 2.96 (bs, 4H*f, i*), and a singlet for methyl protons at δ 2.35 (s, 3H*n*) were observed.

In the IR spectrum of compound (**205**) peaks were observed at 3289 cm^{-1} for N-H stretching and 1653 cm^{-1} for C=O stretching. PMR spectrum of the compound displayed two singlets each representing one proton at δ 10.30 (s, 1H*c*) and 8.06 (s, 1H*b*). Aromatic protons were revealed as two doublets for two thienyl protons at δ 7.44-7.43 (d, *J* = 4.0 Hz, 1H*d*) and 6.94-6.93 (d, *J* = 4.0 Hz, 1H*e*), a multiplet for four protons at δ 6.90-6.83 (m, 4H*j, k, l, m*) and a singlet at δ 6.79 (s, 1H*a*). Two singlets for six methoxy-protons at δ 3.95 (s, 3H*p*) and 3.85 (s, 3Ho), a broad singlet for four methylene protons at δ 3.82 (bs, 4H*g, h*), a singlet for the other methoxy group at δ 3.76 (s, 3H*n*) and broad singlet for four methylene protons at δ 3.08 (bs, 4H*f, i*) were also observed. The mass spectrum of the compound offered a peak at *m/z* 516.3 $[M+H]^+$



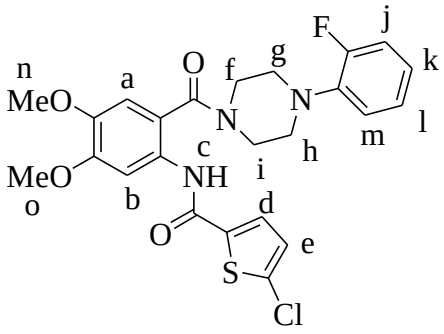
(205)

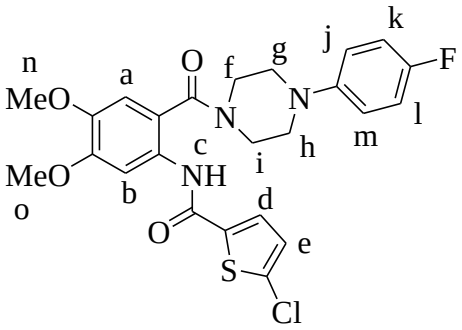


(206)

Compound (**206**) revealed peaks for N-H stretching at 3281 cm^{-1} and C=O stretching at 1655 cm^{-1} in its IR spectrum. PMR spectrum of the compound offered two singlets at δ 10.32 (s, 1H*c*, N*H*CO) and 8.07 (s, 1H*b*). The signals for aromatic protons were seen as a multiplet at δ 7.44 (m, 1H*d*) and doublet at δ 7.04-7.02 (d, *J* = 7.1 Hz, 1H*j*), both peaks signifying one proton with a multiplet for four protons at δ 6.94-6.87 (m, 4H*a, k, l, m*) and a singlet at δ 6.80 (s, 1H*e*). Aliphatic region displayed a singlet for three methoxy-protons at δ 3.95 (s, 3H*p*) and a multiplet for four methylene and other six methoxy-protons merged together at δ 3.87-3.86 (m, 10H*g, h, n, o*) along with a broad singlet at δ 3.08 (bs, 4H*f, i*) for piperazinyl methylene protons. The HR-MS of the compound gave a peak at *m/z* 516.1354 $[M+H]^+$.

Compound (**207**) in its IR spectrum revealed peaks at 3273 and 1650 cm^{-1} for NH and C=O stretching respectively. In its PMR spectrum, signals for compound (**207**) were observed as two singlets for two protons at δ 10.34 (s, 1H*c*, N*H*CO) and 8.14 (s, 1H*b*) and a multiplet at δ 7.45-7.44 (d, *J* = 4.0 Hz, 1H*d*) for a thienyl proton. A doublet representing two aromatic protons at δ 7.09-7.08 (d, *J* = 8.5 Hz, 2H*j, l*), a multiplet for a proton at δ 7.03-7.00 (m, 1H*k*) and a doublet for another thienyl proton at 6.98-6.97 (d, *J* = 4.0 Hz, 1H*e*) were also seen. A multiplet and a singlet for each aromatic proton were noticed at δ 6.94-6.92 (m, 1H*m*) and 6.82 (s, 1H*a*) respectively. Signals at δ 3.98 (s, 3H*o*) as singlet, δ 3.89-3.88 (m, 7H*n, g, h*) as multiplet for seven protons and 3.13 (bs, 4H*f, i*) as broad singlet were also displayed. ^{13}C-NMR of the compound yielded peaks at δ 169.94, 158.94, 151.25, 144.52, 138.32, 136.30, 132.58, 127.62, 127.33, 124.58, 123.41, 119.23, 116.39, 116.23, 113.90, 111.0, 105.96, 56.37, 56.11 and 50.78. The HR-MS of the compound showed peak at *m/z* 504.1155 $[M+H]^+$

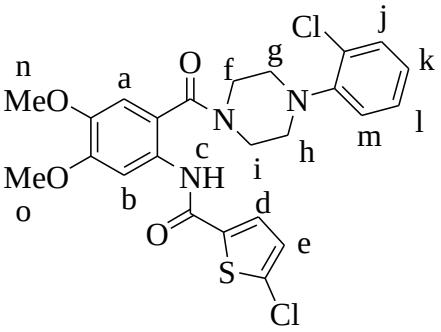




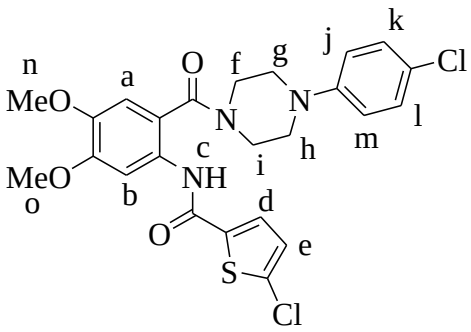
(**207**) **(208)**

Compound (**208**) in its IR spectrum afforded peaks at 3252 cm^{-1} for N-H stretching and at 1656 cm^{-1} for C=O stretching. PMR spectrum of the compound showed peaks at δ 10.34 (s, 1H*c*) and 8.01 (s, 1H*b*) as two separate singlets and δ 7.49-7.48 (d, *J* = 4.0 Hz, 1H*d*) as doublet for a proton. Other aromatic protons appeared at δ 7.01-6.97 (m, 2H*k, l*) as multiplet for two protons, δ 6.96 (d, *J* = 4.0 Hz, 1H*e*) as doublet, δ 6.90-6.87 (m, 2H*j, m*) as multiplet for two protons and δ 6.80 (s, 1H*a*) as a singlet. Among the aliphatic protons, two singlets at δ 3.96 (s, 3H*o*) and 3.86 (s, 3H*n*) for six methoxy-protons and two broad singlets at δ 3.84 (bs, 4H*g, h*) and 3.13 (bs, 4H*f, i*) for methylene protons of piperazine ring were observed.

Compound (**209**) in its IR spectrum displayed bands at 3274 cm^{-1} for N-H stretching and 1649 cm^{-1} for C=O stretching. Its PMR spectrum exhibited peaks at δ 10.34 (s, 1Hc, N*H*CO) and 7.99 (s, 1H*b*) as two singlets along with a doublet for thienyl proton at δ 7.48-7.47 (d, *J* = 4.0 Hz, 1H*d*). A doublet of doublet for one proton at δ 7.38-7.36 (dd, *J* = 1.12, 7.8 Hz, 1H*j*), two multiplets accounting four aromatic protons at δ 7.23-7.19 (m, 1H*l*) and 7.02-6.94 (m, 3H*k, m, e*) and a one proton singlet at δ 6.78 (s, 1H*a*) were observed. Aliphatic protons offered a singlet at δ 3.94 (s, 3H*o*), a multiplet comprising seven protons at δ 3.85 (m, 7H*n g, h*) and a singlet of four methylene protons at δ 3.05 (bs, 4H*f, i*).



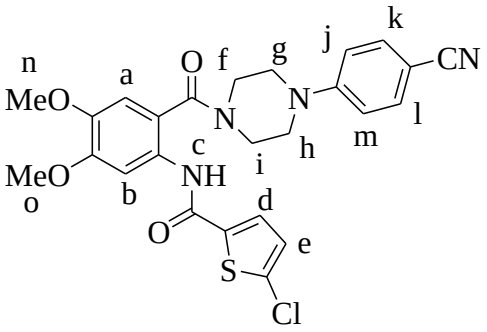
(209)



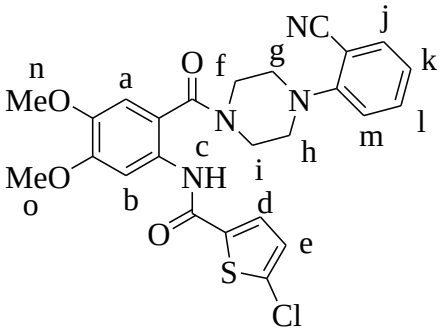
(210)

The IR spectrum of compound (**210**) exhibited signals for NH stretching at 3292 cm^{-1} and C=O stretching at 1654 cm^{-1}. The compound in its PMR spectrum exhibited two singlets for two protons at δ 10.28 (s, 1H*c*, N*H*CO) and 8.05 (s, 1H*b*). Aromatic region afforded four doublets at δ 7.42-7.41 (d, *J* = 4.0 Hz, 1H*d*) for a thiophene proton, δ 7.23-7.21 (d, *J* = 8.8 Hz, 2H*k, l*) for two protons, δ 6.93-6.92 (d, *J* = 4.0 Hz, 1H*e*) singlet for another thiophene proton and δ 6.84-6.82 (d, *J* = 8.8 Hz, 2Hj, m) for two protons. A singlet for an aromatic proton appeared at δ 6.78 (s, 1Ha). The aliphatic protons offered signals at δ 3.94 (s, 3H*o*) and 3.85 (s, 3H*n*) for methoxy and δ 3.82 (bs, 4H*g, h*) and 3.17 (bs, 4H*f, i*) for methylene protons.

In the IR spectrum of compound (**211**) peaks were observed at 2206 cm^{-1} for characteristic CN stretching and 1670 cm^{-1} for C=O stretching. The compound in its PMR spectrum offered signals at δ 9.56 (s, 1H*c*, N*H*CO) and 8.06 (s, 1H*b*) as two singlets and a multiplet representing a proton at δ 7.65-7.64 (m, 1H*d*). A doublet for two aromatic protons at δ 7.54-7.52 (d, *J* = 9.0 Hz, 2H*k, l*), a multiplet at δ 7.35-7.33 (m, 1H*e*), doublet for two aromatic protons at δ 6.87-6.85 (d, *J* = 9.0 Hz, 2H*j, m*) and a singlet at δ 6.76 (s, 1H*a*) were also displayed. Four individual singlets were noticed at δ 3.99 (s, 3H*o*) and 3.88 (s, 3H*n*) for protons of methoxy group and at δ 3.82 (bs, 4H*g, h*) and 3.35 (bs, 4H*f, i*) for piperazinyl protons.



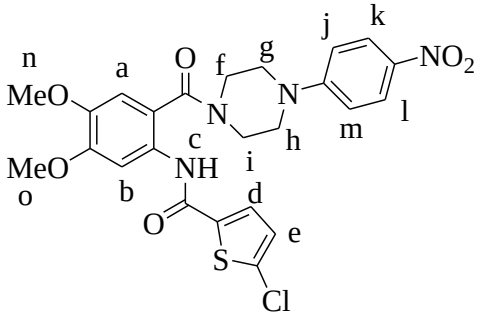
(211)



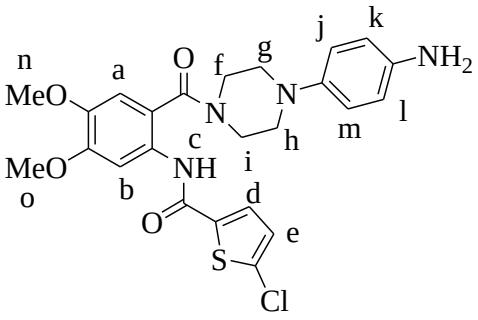
(212)

In its IR spectrum compound (**212**) showed peaks for characteristic CN stretching at 2221 cm^{-1} and 1652 cm^{-1} for C=O stretching. ^{1}H-NMR spectrum of the compound exhibited signals at δ 10.33 (s, 1H*c*, N*H*CO) and 8.09 (s, 1H*b*) as two singlets for two protons, δ 7.61-7.59 (dd, *J* = 1.5, 8.0 Hz, 1H*j*) as doublet of doublet and 7.52-7.49 (m, 1H*l*) as multiplet for a proton. Other aromatic protons showed as doublet at δ 7.43-7.42 (d, *J* = 4.0 Hz, 1H*d*), a multiplet at δ 7.10-7.06 (m, 1H*k*), two separate doublets corresponding to one proton each at δ 6.99-6.98 (d, *J* = 8.0 Hz, 1H*m*) and δ 6.96-6.95 (d, *J* = 4.0 Hz, 1H*e*) with a singlet at δ 6.79 (s, 1H*a*). Aliphatic protons were seen at δ 3.96 (s, 3H*o*), 3.92 (bs, 4H*g, h*), δ 3.88 (s, 3H*n*) and 3.20 (bs, 4H*f, i*) all assigned as four different singlets. ^{13}C-NMR of compound displayed peaks at δ 170.09, 144.58, 136.32, 134.33, 133.93, 132.80, 127.59, 127.32, 122.89, 119.06, 113.68, 111.05, 106.89, 105.99, 56.44. The mass spectrum revealed quasimolecular ion peak $[M+H]^{+}$ at *m/z* 511.4.

The IR spectrum of compound (**213**) displayed signals for C=O stretching at 1668 cm^{-1} and distinguished N-O stretching at 1596 and 1322 cm^{-1}. The PMR spectrum of the compound afforded a singlet at δ 10.34 (s, 1H*c*, -N*H*CO) for amide proton and a doublet for two protons at δ 8.15-8.13 (d, *J* = 9.0 Hz, 2H*k, l*). Signals for other aromatic protons were revealed at δ 8.02 (s, 1H*b*) and 7.46 (s, 1H*d*) as two singlets, δ 6.96-6.95 (d, *J* = 4.5 Hz, 1H*e*) as doublet for thiophene proton and δ 6.89-6.82 (m, 3H*a, j, m*) as multiplet for three protons. Additional protons were seen as singlet at δ 3.97 (s, 3H*o*), as multiplet for seven protons at δ 3.90-3.88 (m, 7H*g, h, n*) and another multiplet for four protons at δ 3.52-3.49 (m, 4H*f, i*) in aliphatic area.



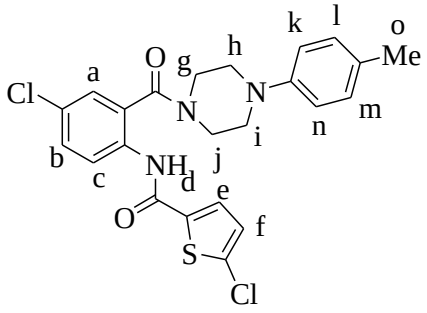
(213)



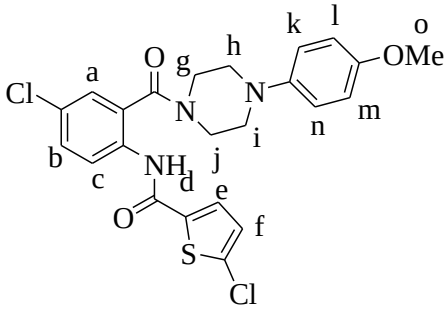
(214)

Compound (**214**) revealed bands at 3435 and 3353 cm^{-1} for NH stretching and 1651 cm^{-1} for C=O stretching in the IR spectrum. ^{1}H-NMR spectrum of the compound displayed peaks at δ 10.33 (s, 1H*c*, N*H*CO) and 8.05 (s, 1H*b*, Ar-*H*) as two singlets, δ 7.47 (s, 1H*d*) as one proton singlet, δ 6.96-6.95 (d, *J* = 4.0 Hz, 1H*e*) as doublet for thienyl proton. A multiplet for three aromatic protons at δ 6.84-6.81 (m, 3H*a, j, m*) and a doublet for two aromatic protons at δ 6.68-6.66 (d, *J* = 9.0 Hz, 2H*k, l*) were also observed. Aliphatic protons were exhibited at δ 3.97 (s, 3H*o*) as singlet, δ 3.87-3.83 (m, 7H*g, h, n*) as a multiplet for methoxy and four methylene protons and δ 3.08 (bs, 4H*f, i*) as a broad singlet.

Peak for C=O stretching at 1658 cm^{-1} was seen in the IR spectrum of compound (**215**). The PMR spectrum of the compound exhibited peaks at δ 10.40 (s, 1H*d*, N*H*CO) as amide proton singlet and a doublet at δ 7.83-7.82 (d, *J* = 4.0 Hz, 1H*e*). A multiplet for two protons at δ 7.53-7.47 (m, 2H*b, c*) and two doublets for two protons at δ 7.42-7.41 (d, *J* = 1.8 Hz, 1H*a*) and 7.14-7.13 (d, *J* = 4.0 Hz, 1Hf) also got displayed with other aromatic protons at δ 7.09-7.04 (m, 2H*l, m*, Ar-*H*) and 6.99-6.89 (m, 2H*k, n*) showing two doublets. Methylene protons were seen at δ 3.73 (s, 2H*h*), 3.48 (s, 2H*i*) and 3.0 (bs, 4H*g, j*) and methyl protons at δ 2.57 (s, 3H*o*).



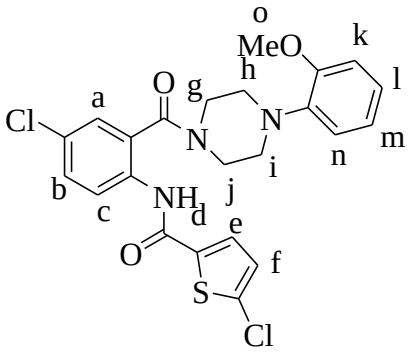
(**215)**



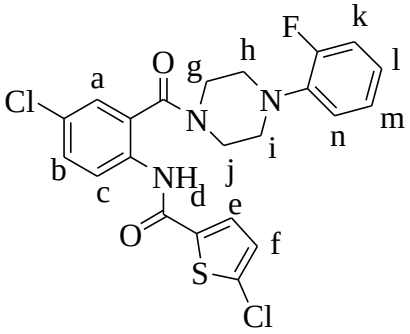
(216)

Compound (**216**) in its IR spectrum showed signals at 3273 cm^{-1} for NH stretching and 1663 cm^{-1} for C=O stretching. PMR spectrum of the compound displayed singlet at δ 9.97 (s, 1H*d*, N*H*CO) and a doublet at δ 8.34-8-32 (d, *J* = 9.0 Hz, 1H*c*). A multiplet for two protons at δ 7.43-7.38 (m, 2H*b, e*) and two doublets each accounting for one proton at δ 7.28-7.27 (d, *J* = 2.5 Hz, 1H*a*) and 6.94-6.93 (d, *J* = 4.0 Hz, 1H*f*) were seen in addition to multiplet for four protons at δ 6.90-6.83 (m, 4H*k, l, m, n*). A multiplet expressing seven protons of methylene and methoxy groups at δ 3.77-3.75 (m, 7H*h, i, o*) and a broad singlet for four protons at 3.09 (bs, 4H*g, j*) got revealed in aliphatic region. ^{13}C-NMR of the compound showed peaks at δ 168.15, 158.86, 154.65, 144.36, 137.76, 136.70, 135.82, 131.19, 128.53, 127.93, 127.56, 127.30, 124.48, 124.10, 119.06, 114.58, 55.53 and 51.24.

Compound (**217**) revealed peaks for NH stretching and C=O stretching at 3224 cm^{-1} and 1658 cm^{-1} respectively in its IR spectrum. In its PMR spectrum compound (**217**) displayed a singlet for an amide proton at δ 10.02 (s, 1H*d*). Aromatic protons showed a doublet at δ 8.29-8.28 (d, *J* = 8.5 Hz, 1H*c*), a multiplet for two protons at δ 7.45-7.41 (m, 2H*b, e*) and a doublet from *meta* coupling at δ 7.30 (d, *J* = 2.5 Hz, 1H*a*). Two additional multiplets for five protons were also noticed at δ 7.08-7.05 (m, 1H*f*) and 6.96-6.90 (m, 4H*k, l, m, n*). Three methoxy and eight piperazinyl methylene protons appeared as singlets at δ 3.90 (s, 3H*o*) and δ 3.77 (bs, 4H*h, i*) with 3.11 (bs, 4H*g, j*) respectively.



(**217**)

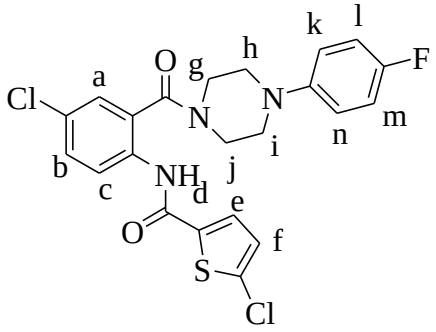


(218)

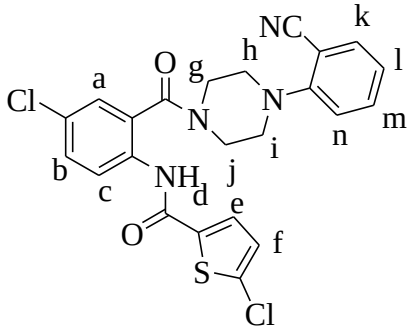
Compound (**218**) conferred peaks at 3274 cm^{-1} for NH stretching and 1651 cm^{-1} for C=O stretching in the IR spectrum. PMR spectrum of the compound gave peaks at δ 9.97 (s, 1H*d*) as a singlet of amidic proton and δ 8.29-8.27 (d, *J* = 8.8 Hz, 1H*c*) as a doublet for one proton. A signal at δ 7.42-7.39 (m, 2H*b, e*) as multiplet for two protons and δ 7.27 (d, *J* = 2.8 Hz, 1H*a*) as

doublet for a proton were also seen. Other aromatic protons afforded two multiplets accounting for five protons at δ 7.08-6.97 (m, 3H*f, k, m*) and 6.93-6.89 (m, 2H*l, n*). Two singlets for methylene protons were observed at δ 3.84 (bs, 4H*h, i*) and 3.11 (s, 4H*g, j*).

The signal for C=O stretching at 1664 cm^{-1} was displayed in the IR spectrum of compound (**219**). The PMR spectrum of the compound offered two singlets at δ 10.35 (s, 1H*d*, N*H*CO) for amide proton and δ 7.78 (s, 1H*a*) for an aromatic proton. A doublet at δ 7.57-7.55 (d, *J* = 8.2 Hz, 1H*c*), multiplet at δ 7.47-7.45 (m, 1H*b*) and two singlets at δ 7.38 (s, 1H*e*) and 7.06 (s, 1H*f*) also appeared. Other aromatic protons revealed a doublet at δ 6.97-6.95 (d, *J* = 7.8 Hz, 2H*l, m*) and a multiplet at δ 6.89 (m, 2H*k, n*). Three broad singlets were offered by piperazinyl methylene protons at δ 3.74 (bs, 2H*i*), 3.49 (bs, 2H*h*) and 3.08 (bs, 4H*g, j*). Peak at *m/z* 478.3 $[M+H]^+$ was noticed in the mass spectrum of the compound.



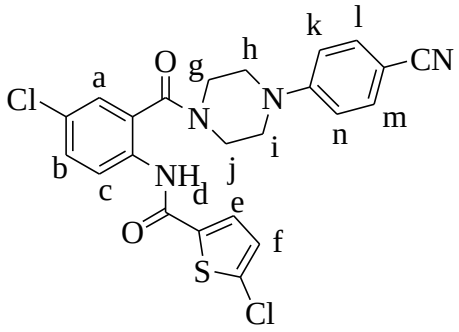
(219)



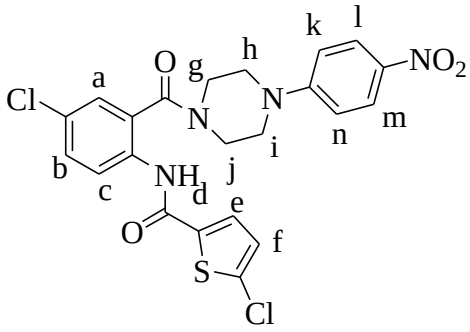
(220)

Compound (**220**) exhibited signals at 3207 cm^{-1} for NH stretching along with CN stretching at 2221 cm^{-1} and 1658 cm^{-1} for carbonyl stretching. The PMR spectrum of the compound afforded singlet for proton of amide at δ 10.01 (s, 1H*d*, N*H*CO), a doublet at δ 8.31-8.29 (d, *J* = 8.5 Hz, 1H*k*) and a doublet of doublet for H*m* proton at δ 7.64-7.62 (dd, *J* = 2.0 and 8.5 Hz, 1H*m*, Ar-*H*). Multiplets for three protons got displayed at δ 7.55-7.52 (m, 1H*c*) and 7.45-7.43 (m, 2H*b, e*). Other peaks for aromatic protons were observed at δ 7.30-7.29 (d, *J* = 2.5 Hz, 1H*a*), 7.03-7.01 (d, *J* = 8.5 Hz, 1H*n*) and 6.96 (d, *J* = 4.0 Hz, 1H*f*) as three doublets and δ 7.13-7.10 (m, 1H*l*, Ar-H) as a multiplet. Broad singlets for methylene protons appeared at δ 3.93 (bs, 4H*h, i*) and 3.25 (bs, 4H*g, j*).

Compound (**221**) in its IR spectrum afforded peaks at 3230 cm^{-1} for NH stretching, 2216 cm^{-1} for noticeable CN stretching and 1658 cm^{-1} for C=O stretching. The PMR spectrum of the compound exhibited amide proton singlet at δ 9.98 (s, 1H*d*, N*H*CO) with two doublets because of *ortho* coupling at δ 8.14-8.12 (d, *J* = 8.8 Hz, 1H*c*) and 7.53-7.51 (d, *J* = 8.8 Hz, 2H*b, e*). Two multiplets for five aromatic protons at δ 7.40-7.37 (m, 2H*l, m*) and 6.89-6.85 (m, 3H*f, k, n*) and a singlet at δ 7.24 (s, 1H*a*) also got revealed. Eight piperazinyl methylene protons offered two singlets at δ 3.83 (bs, 4H*h, i*) and 3.40 (s, 4H*g, j*).



(221)

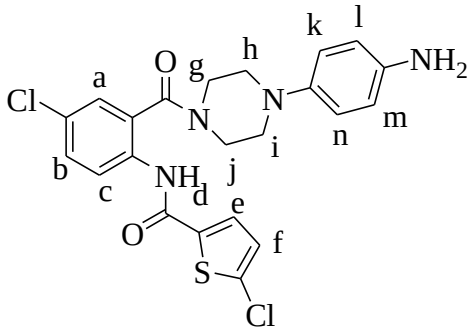


(222)

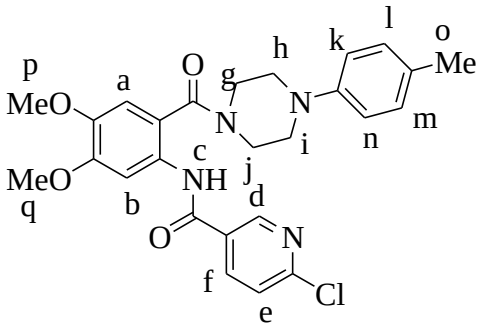
The IR spectrum of compound (**222**) displayed signals at 3206 cm^{-1} for NH stretching, 1647 cm^{-1} for C=O stretching and NO stretching at 1593 and 1323 cm^{-1}. In its ^{1}H-NMR spectrum, the compound showed a singlet at δ 10.03 (s, 1H*d*, N*H*CO) and two different doublets from *ortho* coupling at δ 8.22-8.21 (d, *J* = 9.0 Hz, 1H*c*) and 8.18-8.16 (d, *J* = 7.5 Hz, 2H*l, m*). A multiplet for two protons at δ 7.45-7.42 (m, 2H*a, b*), a doublet at δ 6.94-6.93 (d, *J* = 4.0 Hz, 1H*e*) and a multiplet for three protons at δ 6.87-6.83 (m, 3H*f, k, n*) were also exhibited. Aliphatic protons furnished two broad singlets at δ 3.90 (bs, 4H*h, i*) and 3.54 (bs, 4H*g, j*).

Peaks for characteristic NH stretching at 3447 and 3359 cm^{-1} and C=O stretching at 1662 cm^{-1} were observed in its IR spectra of compound (**223**). Compound (**223**) in its ^{1}H-NMR spectrum showed δ 9.99 (s, 1H*d*) singlet for amidic proton along with a doublet at δ 8.35-8.33 (d, *J* = 9.0 Hz, 1H*c*). A multiplet at δ 7.43-7.42 (m, 1H*e*), a doublet due to *meta* coupling at δ 7.30-7.29 (d, *J* = 2.5 Hz, 2H*a, b*) and a doublet for thienyl proton at δ 6.96-6.95 (d, *J* = 4.0 Hz, 1H*f*) were observed. Two doublets showing *ortho* coupling at δ 6.83-6.82 (d, *J* = 9.0 Hz, 2H*l, m*) and

6.68-6.67 (d, *J* = 9.0 Hz, 2H*k, n*) were also displayed. A multiplet for four aliphatic protons at δ 3.27-3.25 (m, 4H*h, i*) with a broad singlet at δ 3.08 (bs, 4H*g, j*) were noticed.



(223)

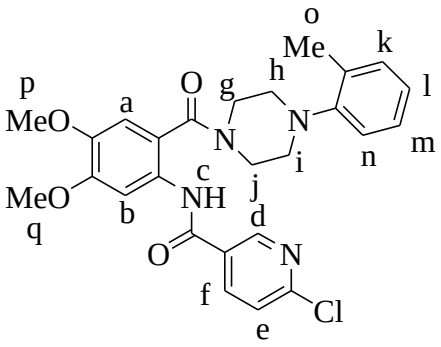


(224)

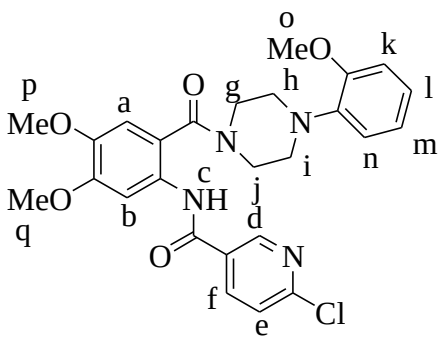
The IR spectrum of compound (**224**) exhibited a band for C=O peak at 1658 cm^{-1}. The PMR spectrum of the compound offered signals as a singlet at δ 10.15 (s, 1H*c,* N*H*CO), a doublet for one proton at δ 8.79 (d, 1H*d, J* = 2.5 Hz) along with a singlet at δ 8.21(s, 1H*b*). A doublet of doublet for H*f* proton because of *ortho* and *meta* coupling with H*e* and H*d* protons respectively was noticed at δ 8.02-8.00 (dd, *J* = 2.5, 9.0 Hz, 1H*f*). A singlet at δ 7.08 (s, 1H*a*), two doublets for two protons each at δ 6.90-6.88 (d, *J* = 8.5 Hz, 2H*l, m*) and 6.83-6.82 (d, *J* = 8.5 Hz, 2H*k, n*) and one proton doublet at δ 6.71-6.69 (d, *J* = 9.0 Hz, 1H*e*) also got displayed in aromatic region. Two singlets for six methoxy-protons at δ 3.98 (s, 3H*q*) and 3.86 (s, 3H*p*) and two multiplets for eight methylene protons at δ 3.86-3.84 (m, 4H*h, i*) and 3.25-3.23 (m, 4H*g, j*) were revealed with a singlet at δ 2.88 (s, 3H*o*). ^{13}C-NMR spectrum of the compound showed peaks at δ 163.97, 151.16, 149.02, 148.64, 148.45, 136.57, 133.28, 129.82, 129.78, 117.05, 116.83, 110.93, 106.19, 105.69, 56.14, 50.36, 49.76, 44.79 and 20.47.

Compound (**225**) showed signals at 3324 cm^{-1} for NH stretching and 1666 cm^{-1} for C=O stretching in its IR spectrum. In its PMR spectrum compound (**225**) showed peaks at δ 10.18 (s, 1H*c,* -N*H*CO) as singlet for amide proton, δ 8.85 (d, *J* = 2.5 Hz, 1H*d*) as a doublet due to meta coupling with H*f* proton and a singlet at δ 8.24 (s, 1H*b*). Other aromatic protons were observed as a doublet at δ 8.06-8.04 (d, *J* = 9.0 Hz, 1H*f*), three multiplets for five protons at δ 7.23-7.21 (m, 2H*k, m*), 7.06-7.04 (m, 2H*l, n*) and 6.99-6.97 (m, 1H*e*), and a singlet for one proton at δ 6.83 (s, 1H*a*). Aliphatic protons were seen at δ 4.01 (s, 3Hq, OCH_3) and 3.90 (s, 3H*p*, OCH_3) as two

singlets for methoxy protons, δ 3.86 (bs, 4H*h, i*) as broad singlet and δ 3.05 (m, 4H*g, j*) as a multiplet for methylene protons, and as a singlet for methyl at δ 2.39 (s, 3H*o*).



(225)

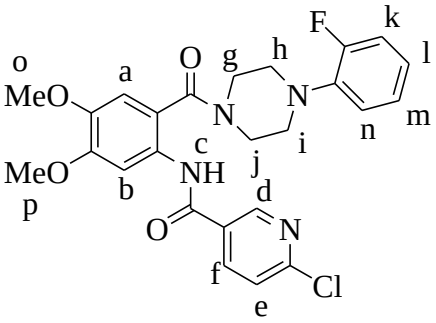


(226)

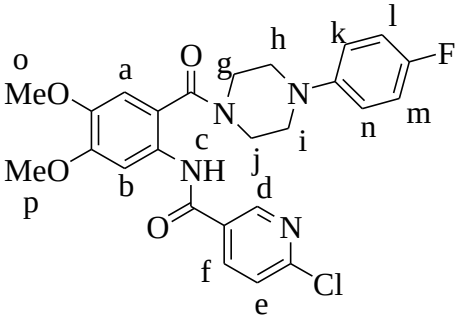
In its IR spectrum compound (**226**) displayed peaks at 3309 cm^{-1} for NH stretching and at 1667 cm^{-1} for C=O stretching. The PMR spectrum of the compound furnished peaks as a singlet at δ 10.13 (s, 1H*c*, N*H*CO) for amidic proton, a doublet at δ 8.81 (d, *J* = 3.0 Hz, 1H*d*) and a singlet at δ 8.20 (s, 1H*b*) each representing one proton. Two multiplets at δ 8.03-8.02 (m, 1H*f*) and δ 7.06-7.01 (m, 4H*k, l, m, n*) for one proton and four protons respectively, were noted along with a singlet at δ 6.80 (s, 1H*a*) and a doublet at δ 6.72-6.70 (d, *J* = 9.0 Hz, 1H*e*). Two singlets for methoxy protons were observed at δ 3.98 (s, 3H*o*) and 3.91 (s, 3H*q*) while two multiplets at δ 3.88-3.86 (m, 7H*h, i, p*) for three methoxy and four methylene protons together, and at δ 3.17 (m, 4H*g, j*) for other methylene protons were also seen. Compound (**226**) in its mass spectrum exhibited quasimolecular ion $[M+H]^+$ peak at *m/z* 511.4.

The C=O stretching peak at 1669 cm^{-1} was seen in its IR spectrum of compound (**227**). The PMR spectrum of the compound revealed a singlet at δ 10.16 (s, 1H*c*, N*H*CO) for amide proton and a doublet due to *meta* coupling for H*d* with H*f* proton at δ 8.99-8.98 (d, *J* = 3.0 Hz, 1H*d*). Other aromatic protons were displayed at δ 8.18 (s, 1H*b*) as a singlet, δ 8.03-8.01 (dd, *J* = 3.0, 9.0 Hz, 1H*f*) as doublet of doublet and δ 7.47-7.46 (d, *J* = 8.5 Hz, 1H*k*) as a doublet for single proton. A multiplet for three protons at δ 7.06-7.04 (m, 3H*l, m, n*) and a singlet at δ 6.82 (s, 1H*a*) along with a doublet at δ 6.73-6.71 (d, *J* = 9.0 Hz, 1H*e*) also appeared. A singlet for

three protons at δ 3.98 (s, 3H*p*), a multiplet for seven protons at δ 3.88-3.87 (m, 7H*h, i, o*) and a multiplet for four methylene protons were seen at δ 3.19 (m, 4H*g, j*) in aliphatic region.



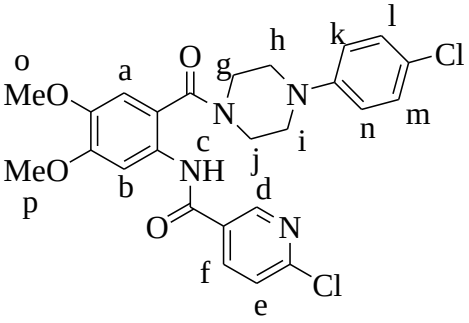
(227)



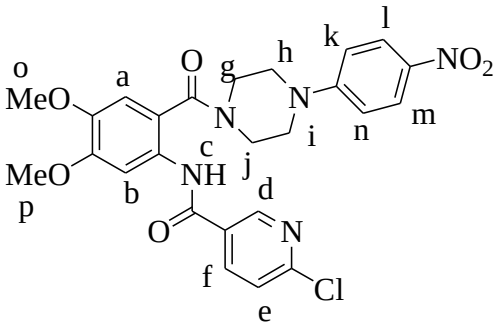
(228)

Compound (**228**) revealed a signal for C=O stretching at 1656 cm^{-1} in its IR spectrum. The proton NMR spectrum of the compound displyed a singlet for amidic proton at δ 10.16 (s, 1H*c*, N*H*CO) and a doublet for pyridyl proton at δ 8.79 (d, *J* = 2.5 Hz, 1H*d*). A singlet at δ 8.21 (s, 1H*b*), a doublet of doublet at δ 8.03-8.01 (dd, *J* = 2.5, 9.0 Hz, 1H*f*) and a singlet at δ 6.99 (s, 1H*a*) each peak accounting for one proton were observed. Two multiplets for four protons at δ 6.98-6.93 (m, 2Hl, *m*) and 6.88-6.85 (m, 2H*k*, *n*) and a doublet at δ 6.72-6.70 (d, *J* = 9.0 Hz, 1H*e*) were also seen. Aliphatic protons appeared at δ 3.98 (s, 3H*p*) and 3.87 (s, 3H*o*) as two singlets for methoxy protons and δ 3.84 (m, 4H*h, i*) and 3.21 (m, 4H*g, j*) as two multiplets for piperazinyl methylene protons. The mass spectrum of the compound gave a $[M+H]^+$ peak at *m/z* 499.4.

Compound (**229**) in the IR spectrum showed signals at 3301 and 1660 cm^{-1} for NH and C=O stretching respectively. PMR spectrum of the compound offered a singlet for proton of amide at δ 9.06 (s, 1H*c*). Aromatic region showed a doublet at δ 8.29-8.27 (d, *J* = 8.4 Hz, 1H*d*), a singlet at δ 7.53 (s, 1H*b*), a multiplet for three protons at δ 7.25-7.22 (m, 3H*l*, *m*, *n*), a singlet at δ 7.04 (s, 1H*a*, Ar-*H*) a doublet for two protons at δ 6.89-6.87 (d, *J* = 8.4 Hz, 2H*e, f*) and a multiplet for a proton at δ 6.73-6.70 (m, 1H*k*). A multiplet corresponding to six protons at δ 4.03-3.99 (m, 6H*o, p*) and two broad singlets accounting eight methylene protons at 3.88 (bs, 4H*h, i*) and 3.28 (bs, 4H*g, j*) were also observed. The HR-MS spectrum of the compound revealed a signal at *m/z* 515.1247 $[M+H]^+$.



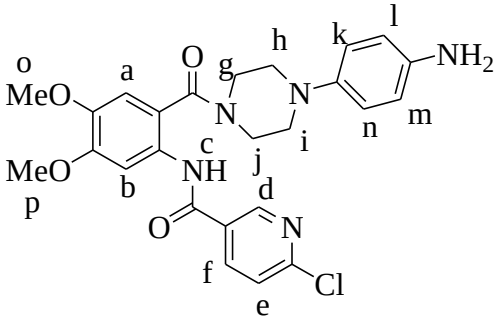
(**229**)



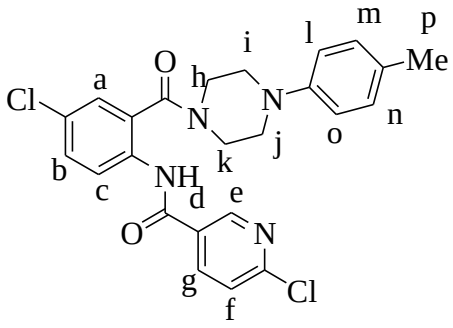
(230)

Compound (**230**) exhibited peaks at 1658 cm^{-1} for C=O stretching and at 1516 and 1324 cm^{-1} for characteristic NO stretching. ^{1}H-NMR spectrum of the compound gave signals at δ 10.23 (s, 1H*c*, N*H*CO) as a singlet of amide proton and a doublet for pyridyl proton at δ 8.99-8.98 (d, *J* = 2.5 Hz, 1H*d*). Other aromatic protons displayed a singlet at δ 8.82 (s, 1H*b*), a multiplet at δ 8.20-8.18 (m, 3H*l, m, n*) and doublet of doublet at δ 8.08-8.06 (dd, *J* = 2.5, 9.0 Hz, 1H*f*) in addition to δ 7.49-7.47 (d, *J* = 8.5 Hz, 1H*k*) as a doublet and δ 6.85-6.84 (m, 2H*a, e*) as multiplet. Aliphatic protons were noticed at δ 4.02 (s, 3H*p*) as singlet and δ 3.90-3.88 (m, 7H*h, i, o*) and 3.64 (m, 4H*g, j*) as two separate multiplets.

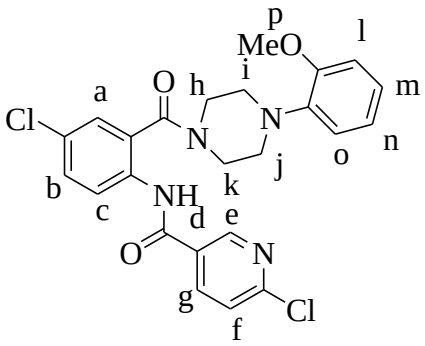
Signals for NH stretching and C=O stretching were seen at 3361 and 1667 cm-1 respectively in the IR spectrum of compound (**231**) while its PMR spectrum offered two singlets at δ 10.63 (s, 1H*c*, N*H*CO) and 9.01 (s, 1H*b*). Additional peaks got revealed at δ 8.20-8.18 (m, 2H*d, f*) as two proton multiplet, δ 7.49-7.47 (d, *J* = 8.0 Hz, 1H*e*) as a doublet, δ 6.85-6.82 (m, 2H*k, n*) and 6.69-6.67 (m, 2H*l, m*) as two multiplets for two protons each and a singlet at δ 6.74 (s, 1H*a*). A broad singlet for two amino protons at δ 4.28 (bs, 2H*q*, NH_2) was observed. Six methoxy-protons as two singlets at δ 4.01 (s, 3H*p*) and 3.90 (s, 3H*o*) along with a multiplet at δ 3.22-3.13 (m, 4H*h, i*) and broad singlet at δ 3.10 (bs, 4H*g, j*) were also seen.



(231)



(**232)**



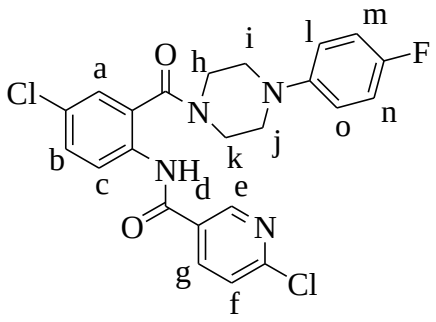
(233)

Compound (**232**) exhibited peaks for NH stretching at 3294 cm^{-1} and C=O stretching at 1673 cm^{-1} in its IR spectrum. ^{1}H-NMR spectrum of the compound showed singlet at δ 9.82 (s, 1H*d*, N*H*CO), a doublet due to *meta* coupling at δ 8.76-8.75 (d, *J* = 2.5 Hz, 1H*e*) and a doublet expressing *ortho* coupling at δ 8.46-8.44 (d, *J* = 9.0 Hz, 1H*c*. Other aromatic protons afforded two doublets of doublet for two different protons at δ 8.0-7.97 (dd, *J* = 2.5 and 9.0 Hz, 1H*g*) and 7.44-7.42 (dd, *J* = 2.5 and 8.5 Hz, 1H*b*). Four individual doublets at δ 7.27-7.26 (d, *J* = 2.5 Hz, 1H*a*), 6.90-6.88 (d, *J* = 8.5 Hz, 2H*m, n*), 6.84-6.82 (d, *J* = 8.5 Hz, 2H*l, o*) and 6.70-6.68 (d, *J* = 9.0 Hz, 1H*f*) were observed. Aliphatic protons were noticed as two multiplets at δ 3.85 (m, 4H*i, j*) and 3.24 (m, 4H*h, k*), and a singlet at δ 2.28 (s, 3H*p*). Mass spectrum of the compound exhibited quasimolecular ion peak at *m/z* 469.4 $[M+H]^{+}$

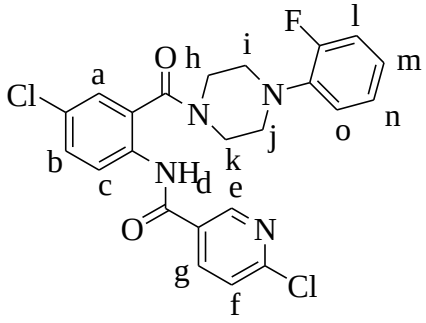
The IR spectrum of compound (**233**) showed signal for C=O stretching at 1670 cm^{-1} The PMR spectrum of the compound displayed a singlet due to amide proton at δ 9.81 (s, 1H*d,* N*H*CO) and another singlet for pyridyl proton at δ 8.77 (s, 1H*e*). Three doublets each accounting

for one proton were noticed at δ 8.46-8.44 (d, *J* = 8.8 Hz, 1H*c*), 8.00-7.98, (d, *J* = 9.2 Hz, 1H*g*) and 7.43-7.40 (d, *J* = 9.2 Hz, 1H*b*). Other aromatic protons showed a singlet at δ 7.25 (s, 1H*a*), a multiplet for four protons at δ 6.95-6.88 (m, 4H*l, m, n, o*) and a doublet at δ 6.71-6.68 (d, *J* = 9.2 Hz, 1H*f*). Two singlets at δ 3.90 (s, 4H*i, j*) and 3.86 (s, 3H*p*) for methylene and methoxy protons respectively were seen with a singlet for other methylene protons at δ 3.17 (m, 4H*h, k*). In its mass spectrum, compound furnished $[M]^+$ peak at *m/z* 484.2

Compound (**234**) afforded peaks at 3298 cm^{-1} for NH stretching and 1675 cm^{-1} for C=O stretching in its IR spectrum. PMR spectrum of the compound exhibited peaks at δ 9.84 (s, 1H*d,* N*H*CO) as singlet for amide proton, two doublets each corresponding to one proton at δ 8.76-8.75 (d, *J* = 2.5 Hz, 1H*e*) and 8.45-8.43 (d, *J* = 9.0 Hz, 1H*c*), two doublets of doublet at δ 8.01-8.00 (dd, *J* = 2.5, 9.0 Hz, 1H*g*) and 7.45-7.44 (dd, *J* = 2.5, 9.0 Hz, 1H*b*). A doublet for one proton showing *meta* coupling at δ 7.27-7.26 (d, *J* = 2.5 Hz, 1H*a*), a multiplet of four protons at δ 6.93-6.87 (m, 4H*l, m, n, o*) and a doublet at δ 6.71-6.69 (d, *J* = 9.0 Hz, 1H*f*) were displayed. Two multiplets accounting for four protons each of piperazine ring were observed at δ 3.84 (m, 4H*h, k*) and 3.20 (m, 4H*i, j*). Carbon NMR spectrum of the compound exhibited peaks at δ 168.20, 163.85, 160.24, 158.43, 156.77, 156.52, 148.36, 136.74, 136.37, 131.09, 128.08, 127.36, 124.33, 118.67, 118.28, 115.83, 115.74, 115.65, 115.56, 105.67, 50.77, 50.14 and 44.72.



(234)



(235)

Compound (**235**) revealed in its IR spectrum signals at 1673 cm^{-1} for C=O stretching while its PMR spectrum showed a singlet at δ 9.84 (s, 1H*d,* N*H*CO) and a doublet because of *meta* coupling at 8.76 (d, *J* = 2.4 Hz, 1H*e*). A doublet at δ 8.30-8.28 (d, *J* = 9.2 Hz, 1H*c*) and a doublet of doublet at 8.01-8.00 (dd, *J* = 2.4, 9.0 Hz, 1H*g*) both for one proton, two multiplets for

four protons at δ 7.44-7.41 (m, 2H*l*, *n*) and 6.93-6.89 (m, 2H*m*, *o*), and two doublets at δ 7.29-7.28 (d, *J* = 2.4 Hz, 2H*a*, *b*) and 6.70-6.68 (d, *J* = 9.2 Hz, 1H*f*) were observed in aromatic region. Aliphatic protons gave two multiplets for methylene protons at δ 3.86 (m, 4H*i*, *j*) and 3.19 (m, 4H*h*, *k*).

4.1.2 Biological Evaluation

4.1.2.1 *In vitro* FXa enzyme inhibition study

All the target compounds were preliminarily tested for their antithrombotic activity by *in vitro* FXa enzyme inhibition chromogenic assay [4] using rivaroxaban as a positive control. Most of them exhibited more than 50 % inhibition at 20 µM concentration (**Figure 4.1** to **4.3**). However, only those compounds showing > 60% inhibition (total 20 compounds) were selected for determination of their IC_{50} values. The results revealed that several compounds from the series displayed good to moderate inhibitory activities against FXa enzyme with IC_{50} values ranging from 0.6 to 39 µM. Compounds (**201**) and (**208**) were found to be the most active compounds having IC_{50} values of 0.61 and 0.74 µM respectively (**Table 4.3**).

To check the selectivity of the inhibitors for FXa over thrombin, a thrombin specific chromogenic assay was also performed in which negligible inhibition (< 10%) of thrombin was observed for all the synthesized compounds.

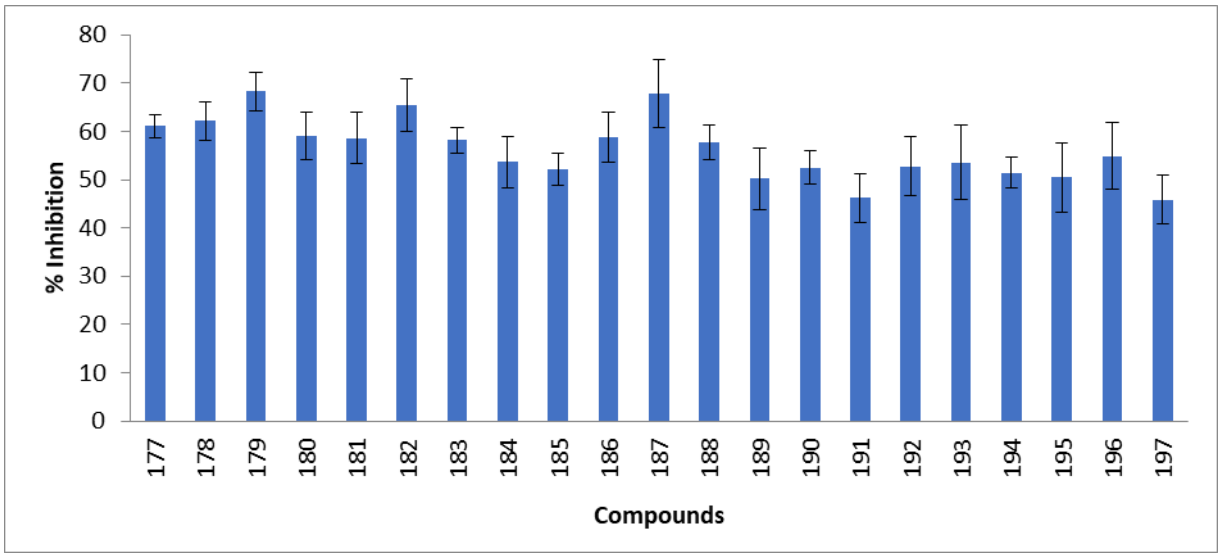


Figure 4.1: *In vitro* FXa inhibition activity of 4-chlorophenyl derivatives (**177-197**)

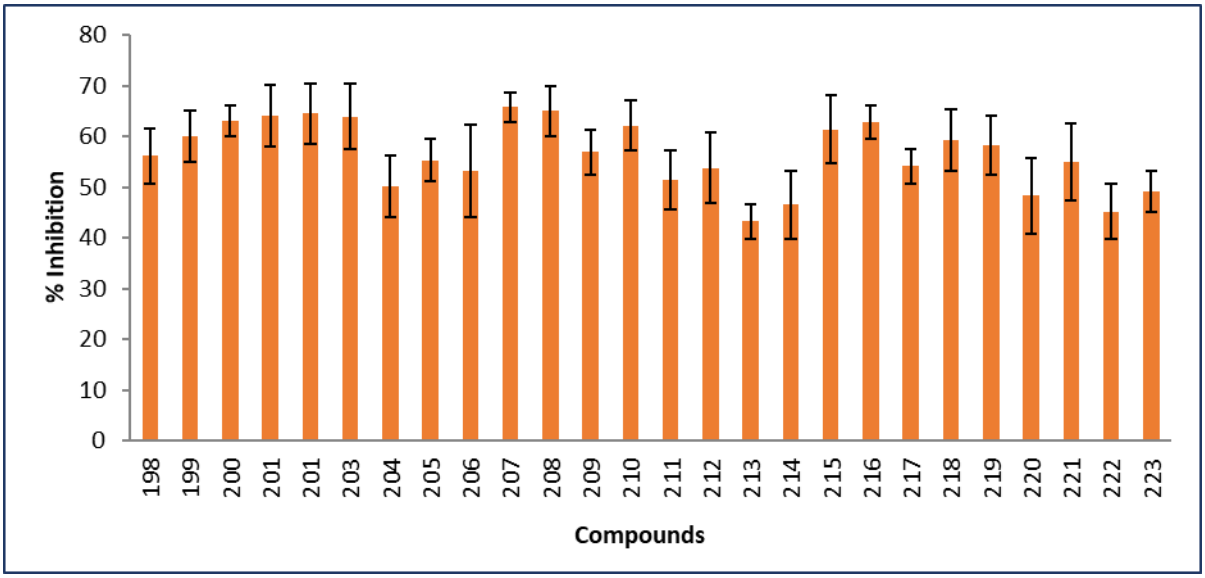


Figure 4.2: *In vitro* FXa inhibition activity of 5-chlorothiophenyl derivatives (**198-223**)

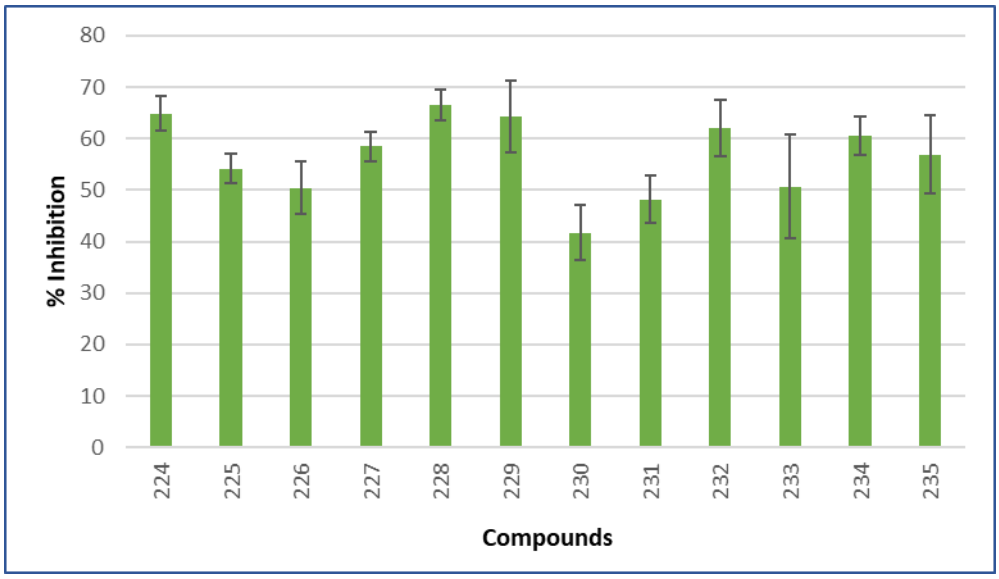


Figure 4.3: *In vitro* FXa inhibition activity of 6-chloropyridyl derivatives (**224-235**)

Table 4.3: IC_{50} values of selected compounds for FXa inhibitory activity.

Compound	FXa IC_{50} (µM)	Compound	FXa IC_{50} (µM)
177	31.7 ± 3.2	**207**	8.7 ± 1.3
178	26.6 ± 3.7	**208**	**0.74 ± 0.14**
179	14.3 ± 2.6	**210**	9.2 ± 2.1
182	18.6 ± 3.2	**215**	25.6 ± 6.3
187	**6.3 ± 1.4**	**216**	35.2 ± 5.8
199	25.2 ± 4.3	**224**	**1.8 ± 0.3**
200	19.9 ± 4.5	**228**	8.09 ± 1.6
201	**0.61 ± 0.07**	**229**	22.2 ± 4.1
202	15.3 ± 2.7	**232**	39.9 ± 8.4
203	10.7 ± 1.6	**234**	23.8 ± 4.7
Rivaroxaban	0.046 ± 0.008		

The results are expressed in mean ± SEM (n = 3).

4.1.2.2 *Ex vivo* prothrombin time (PT) and activated partial thromboplastin time (aPTT) prolongation in rats

The target compounds displaying potent inhibitory activity were further evaluated for their anticoagulation potential by *ex vivo* prothrombin time (PT) and activated partial thromboplastin time (aPTT) prolongation in rats.[5] PT and aPTT are usually measured to check the effect of an inhibitor on extrinsic and intrinsic pathways of coagulation respectively and thus signify efficacy of anti-thrombotic agents. The activity was determined in rats after 2 hrs of oral administration of the inhibitors at a dose of 30 mg/kg. The results (**Table 4.4**) demonstrated that compounds (**201**) (19.9 and 40.8 sec.) and (**224**) (13.6 and 40.0 sec.) showed higher prolongation in both PT and aPTT compared to the standard rivaroxaban (11.4 and 37.9 sec.). Other compounds like (**200**), (**203**) and (**208**) also displayed comparable activity to the standard drug.

Table 4.4: *Ex vivo* PT and aPTT prolongation in rats

Compound	PT (sec.)	aPTT (sec.)	Compound	PT (sec.)	aPTT (sec.)
177	9.8	22.7	**208**	11.6	25.3
178	11.2	25.2	**210**	10.8	25.6
179	10.4	28.5	**215**	8.6	25.9
182	9.3	35.3	**216**	8.8	24.2
187	9.3	27.7	**224**	**13.6**	**40.0**
199	10.3	28.5	**228**	9.5	23.7
200	11.8	30.4	**229**	7.9	23.3
201	**19.9**	**40.8**	**232**	9.6	29.8
202	10.5	32.6	**234**	10.1	28.6
203	**13.7**	27.6	**Rivaroxaban**	**11.4**	**37.9**
206	9.9	18.5	**Vehicle**	8.7	25.3

4.1.2.3 *In vivo* bleeding time in rats

To assess the hemorrhagic properties caused by the target compounds, bleeding times were measured.[6] The test results are depicted in **Table 4.5** comparing the bleeding time of the most active compounds of the series, (**187**, **201**, **208** and **224**) and standard rivaroxaban. The data revealed that bleeding times were not significantly different from the standard drug in rats at the antithrombotic doses (5 mg/kg and 10 mg/kg) except for compound (**187**) which exhibited a dose-dependent increase in bleeding time. Compound (**201**) displayed a safety profile similar to the standard drug rivaroxaban.

Table 4.5: *In vivo* bleeding time in rats

Compound	Bleeding time (sec.) at doses of	
	5 mg / kg	10 mg / kg
187	162 ± 5.8**	275 ± 13.7**
201	**116 ± 3.6****	**192 ± 9.6****
208	138 ± 4.3**	224 ± 11.2**
224	126 ± 4.0**	207 ± 10.3**
Rivaroxaban	**115 ± 2.4****	**188 ± 9.4****
Control	76 ± 2.1	

All values indicated as mean ± S.E.M.

Different anthranilamide analogs containing substituted 4-phenylpiperazinyl group as P4 fragment and haloaromatic ring as P1 motif were evaluated for FXa inhibition activity. In a series of 58 compounds, various substituents e.g. –Cl, -F, -CH_3, -OMe, -CN, -NO_2 and –NH_2 at *ortho* and *para* positions of the phenylpiperazinyl moiety were incorporated to obtain a spectrum of anti-FXa potencies. Cyano, nitro and amino derivatives showed 41-55% inhibition in a primary screening assay while fluoro, methyl and chloro derivatives displayed > 60% inhibition (**Figures 4.1-4.3**). Some methyl derivatives (**224**, IC_{50} = 1.8 µM; **203**, IC_{50} = 10.7 µM) were found to have moderate biological activities. Compounds (**201**) and (**208**) containing 4-fluorophenylpiperazine as P4 surrogate and 5-chlorothiophene as P1 moiety exhibited the highest potency with IC_{50} values of 0.61 and 0.74 µM respectively. This could be because of favorable physicochemical properties and selectivity provided by these two moieties for FXa inhibitory activity. The overall order followed by the substituents present in phenylpiperazine moiety was, 4-F > 4-CH_3 > 4-Cl > 4-OMe for anti-FXa activity. An activity difference between the halo substituents was also observed in P4 side chain where *p*-fluoro analogs (**201** and **208**) were found more active than the corresponding *p*-chloro derivatives (**202** and **210**). This was true for all the isomeric compounds (e.g. **178** vs. **187**; **207** vs. **208**) and was also followed by rest of the compounds of the series irrespective of other substituents. It is worth noting that compound (**201**) exhibited better anticoagulant activity by prolongation in prothrombin time (**201**, PT = 19.9 sec.) than the reference drug rivaroxaban (PT = 11.4 sec.). Though compound (**201**) was

about one-tenth as potent as rivaroxaban in the *in vitro* assay, this could be due to better bioavailability of compound (**201**) in the whole animal model in comparison to rivaroxaban. Interestingly compound (**201**) also displayed similar safety profile as rivaroxaban in bleeding risk evaluation in rats.

Very well explored anti-FXa neutral substituents viz. methoxy and chloro groups were used at 5-position of the central phenyl ring. 5-Methoxy derivatives (**179, 200, 201, 202**) exhibited better potency than 5-chloro compounds (**215, 216, 232, 234**). Introduction of another methoxy group at C4-position (4, 5-dimethoxy substituents) on phenyl ring improved the inhibitory potency (**187, 208, 224**) with a few exceptions. The general order of activity in the series was 4,5-dimethoxy > 5-methoxy > 5-chloro.

In case of P1 fragments on anthranilamide scaffold, highly explored 4-chlorobenzene, 5-chlorothiophene and 6-chloropyridine S1 binding ligands were incorporated for FXa binding affinity and selectivity. Compounds (**201** and **208**) with 5-chlorothiophene moiety exhibited the most potent anti-FXa activity. For the other P1 surrogates, compound (**187**; IC_{50} = 6.3 µM) with 4-chlorophenyl ring and compound (**224**; IC_{50} = 1.8 µM) containing 6-chloropyridine were the most active agents. The observed overall order of FXa inhibitory potency for P1 substituents was 5-chlorothiphene > 6-chloropyridine > 4-chlorobenzene.

4.1.4 Molecular docking and dynamics simulation studies[7-10]

Computational studies were carried out to gain insight into the interaction of the newly synthesized compounds with FXa enzyme. The crystal structure of FXa-rivaroxaban complex showed bindings of ligand in the active site with the chlorothiophene moiety in the S1 pocket and morpholinone moiety in the S4 pocket of FXa. In the docking studies rivaroxaban and the newly synthesized compounds were found to bind in the same pocket with the chlorothiophene /chlorobenzamide moiety of the active compounds (**187**, **201**, **203**, **208** and **224**) resting in the S1 pocket and substituted phenyl piperazine moiety in S4 pocket. The docking scores are mentioned in **Table 4.6** indicating high structural affinity of the synthesized ligands for FXa. On visualization it was observed that the ligands were overlaying on the top of rivaroxaban within the active site. **Figure 4.4a** showed the most potent molecule (**201**) overlaid with rivaroxaban in the active site of FXa. Rest of the compounds with promising biological activities also occupied

the same region. The sulphur atom of thiophene ring showed a side chain acceptor interaction with Ser185 (length 3.39 Å) further burying it in the S1 pocket and stabilizing the interaction. The 4-methoxyphenyl central ring is more solvent exposed compared to the rest of the ligand and the 4-fluorophenyl group attached to the piperazine ring forms an arene-hydrogen π-interaction with Tyr85 residue (**Figure 4.4a**).

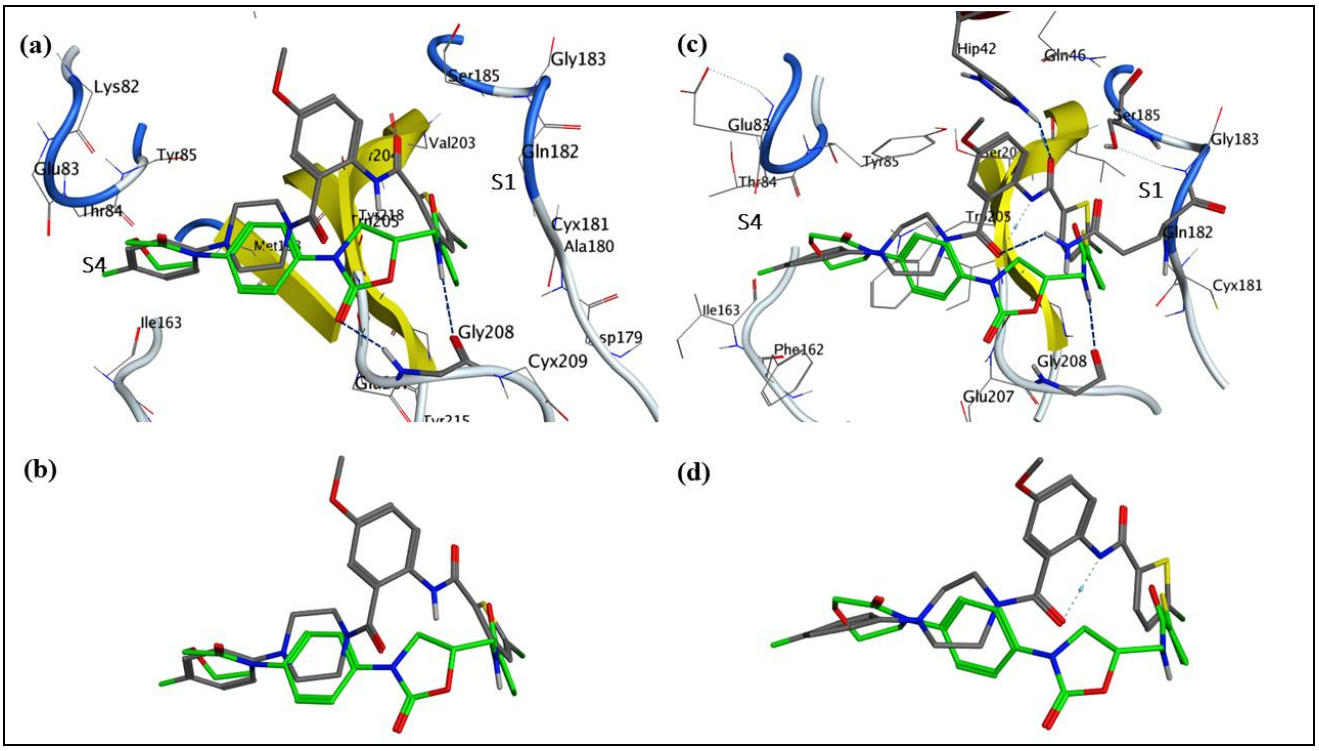
To further evaluate the stability of the complexes of FXa with rivaroxaban, (**201**) and rest of the ligands, a post-docking molecular dynamics simulation was performed. Each protein-ligand simulation was performed for 100 ns in explicit solvent model at salt concentration of 0.15M. Post-docking molecular dynamics study of the newly synthesized compounds and the standard drug provided a deeper understanding of the binding and affinity of these compounds to the target enzyme. At the end of the simulation, the molecules were analyzed for their stability and interactions with the residues of the active site. Compound (**201**) showed formation of two hydrogen bonds (**Figure 4.4c**). Both the carbonyl oxygens formed respective hydrogen bonds; O1 (piperazine carbonyl) formed hydrogen bond with Gln182 (bond length 2.92 Å) and O2 (thiophene carbonyl) formed side chain hydrogen bond with His42 (bond length 2.87 Å). The hydrogen bond between sulphur of thiophene ring and Ser185 was found intact with reduction in bond length to 3.05 Å. The molecular dynamics simulations of the enzyme-ligand complex trajectories were processed to analyze the RMSD of the protein and the ligands. The graph in **Figure 4.4e** shows the ligand RMSD; FXa-rivaroxaban (black) varies to a small extent by 2-3 Å between 10 to 15 and around 70 ns after which the complex is stable for rest of the simulation period. The reason behind these fluctuations is the ligand conformational changes taking place during simulation. The RMSD for the FXa-**201** (Yellow colored) rises from 0-2 Å during first 20 ns and later lowers and stabilizes to 1 Å towards the end of the simulation period indicating enhanced stability of this complex. To analyze the effect of protein fluctuations during simulation we studied the fluctuations per residue, and the RMSF plot for each residue is presented in **Figure 4.4f**. The RMSF plot showed high fluctuation in the region between the residues 50 to 55, however these residues belong to the other lobe of the protein and has no direct impact on the binding of the ligands. RMSF in the active site is between 0 to 1.5 Å, which is considered as sufficiently stable.

To study the binding free energies of these complexes a Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) analysis was performed on the simulation trajectories. The contribution of various factors is detailed for the standard drug and other ligand-enzyme complexes. The binding free energy (ΔG_{bind}) between the ligand (L) and the receptor (R) to form a complex RL is calculated as (**i**), where ΔE_{MM} represents the gas-phase molecular mechanics energy which includes bond stretching, angle bending, torsion rotation, van der Waals and electrostatic contributions as given in (**ii**)

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{pol} + \Delta G_{np} - T\Delta S \quad \textbf{(i)}$$

$$\Delta E_{MM} = \Delta E_{bond} + \Delta E_{angle} + \Delta E_{tors} + \Delta E_{vdw} + \Delta E_{elec} \quad \textbf{(ii)}$$

The ΔG_{pol} term represents the polar contribution to the solvation free energy, ΔG_{np} term stands for the non-polar contributions and the ΔS term represents conformational entropy estimated by normal mode analysis. The gas-phase interaction energy (ΔE_{MM}) between the receptor and the ligand is the sum of van der Waals (ΔE_{vdw}) and electrostatic (ΔE_{elec}) interaction energies. Herein, we calculated the GB energies for all the complexes. It was observed that ΔG_{bind} for FXa-rivaroxaban was -37.32 kcal/mol^{-1} and for that of FXa-**201** complex was -38.18 kcal/mol^{-1}. This is consistent with the results obtained from molecular docking studies. The ΔE_{VDW} in case. of FXa-rivaroxaban is higher (-52.35 kcal/mol^{-1}) compared to FXa-**201** (-48.95 kcal/mol^{-1}) suggesting higher contribution of van der Waals interactions. However, the electrostatic energy calculated by the MM force field is higher for the FXa-rivaroxaban compared to FXa-**201**. The GB contribution to FXa-rivaroxaban is slightly higher than FXa-**201**. The energy contribution due to solvent accessible surface area (ΔG_{Surf}) is higher in case of FXa-**201** complex, providing higher stability to the complex and hence a better ΔG_{bind} is observed. The individual contributions from MM and GB calculations suggested that both the complexes (ie. FXa-rivaroxaban and FXa-**201**) are almost equally stable in terms of binding free energies.


(a)
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Glu83
Thr84
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Ile163
Ser185
Gly183
Val203
Gln182
S1
Cyx181
Ala180
Gly208
Cyx209
(b)
(c)
Hip42
Gln46
Glu83
Tyr85
Thr84
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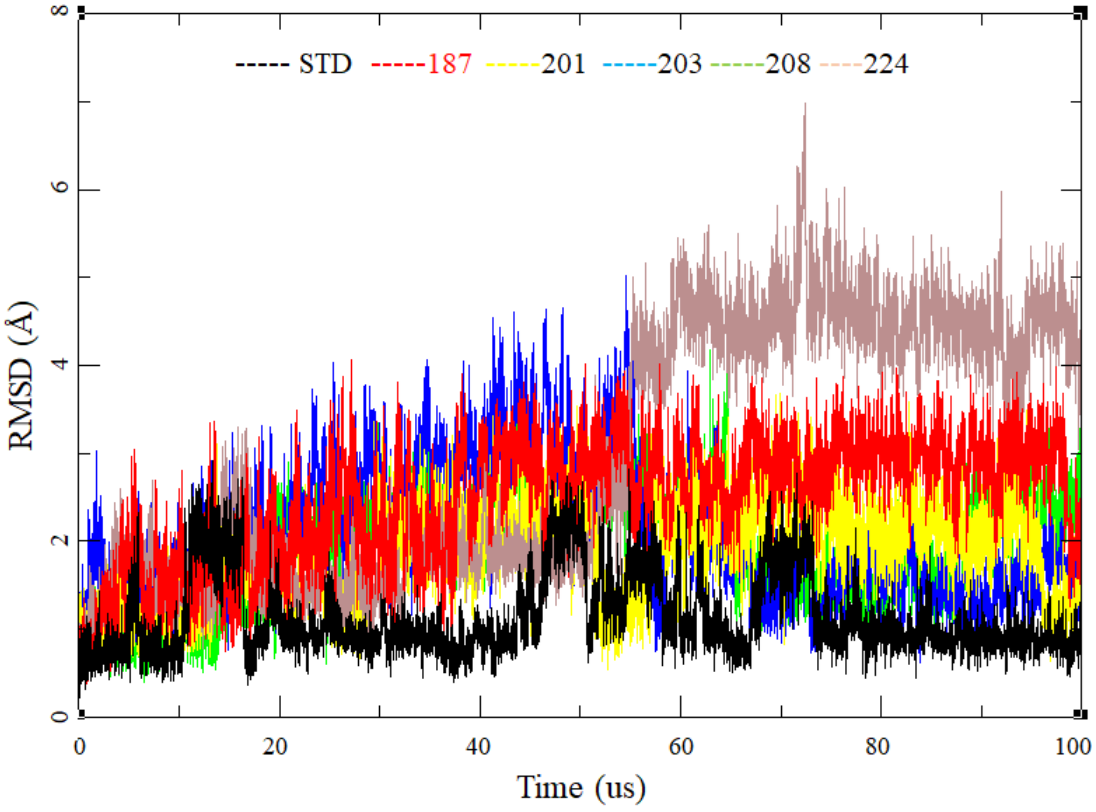
(d)


----- STD -----187 -----201 -----203 -----208 -----224
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4
6
8
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20
40
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80
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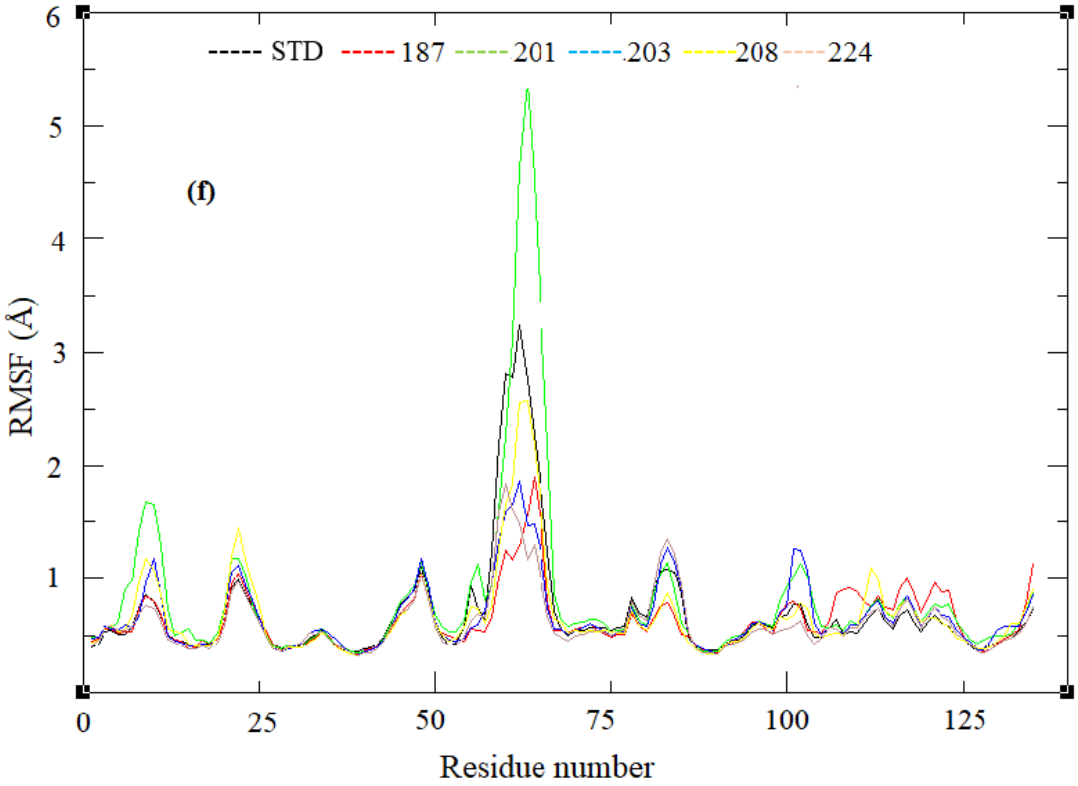


Figure 4.4: Molecular docking and molecular dynamics simulation studies **(a)** Docking pose for FXa-rivaroxaban (green) and overlay of (**201**) (Element color); **(b)** Molecular overlay of rivaroxaban and (**201**); **(c)** Post-docking molecular dynamics simulation pose of (**201**) at the end of 100 ns simulation of the protein-ligand complex; **(d)** Molecular overlay of rivaroxaban and (**201**) after post-docking molecular dynamics simulation; **(e)** Ligand RMSD for rivaroxaban, (**187**), (**201**), (**203**), (**208**) and (**224**) during molecular dynamics simulation for 100ns; **(f)** RMSF of FXa residues in individual complexes during molecular dynamics simulation for 100ns.

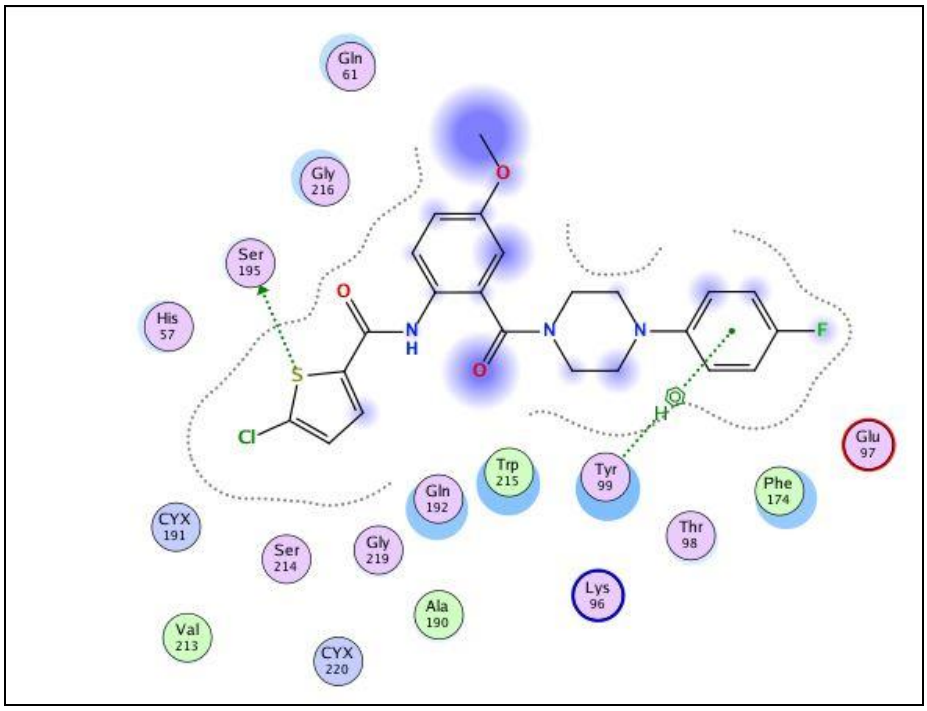


Figure 4.5: 2D interaction map for compound (**201)** with FXa after molecular dynamics simulation.

Table 4.6: Docking score (ChemGauss4) and computed MM/GBSA binding free energy for the predicted pose of ligand bound to FXa receptor proteins. Standard deviations in parentheses. Energies in kcal/mol.

Complexes	**Dock Score**	**ΔG_{bind}** kcal/mol
Rivaroxaban	-9.39	-37.32 (3.8)
187	-10.57	-37.41 (3.7)
201	-11.87	-38.18 (4.3)
203	-8.60	-34.89 (4.5)
208	-8.97	-34.98 (4.0)
224	-10.10	-31.15 (4.8)

4.1.5 Prediction of Physicochemical and Pharmacokinetic properties

Theoretical physicochemical and pharmacokinetic properties of the synthesized compounds and standard rivaroxaban were calculated by pkCSM[11] (**Table 4.7**). The physicochemical properties like molecular weight (474.10–503.98 Da), hydrogen bond donors (1), acceptors (5–6), hydrophobicity (4.27–4.94), polar surface area (193.71–208.96) and number of rotatable bonds (5–6) were all in the acceptable ranges. The synthesized compounds are intended for oral route of administration and hence the gastrointestinal absorption is an important factor. The predicted values for intestinal absorption displayed a promising trend in this series with all the compounds having similar absorption pattern to rivaroxaban. Experimentally rivaroxaban is absorbed >80 % via gastrointestinal route which corroborates to the theoretical value of 92.80 %. A compound is considered to have Caco-2 permeability if it has a Papp >8 x 10^{-6} cm/s, in the pkCSM predictive model. The Caco2 permeability of rivaroxaban is 8.9 x 0.1 10^{-6} cm/s at concentration of 0.92 µM, which is reflected in the theoretical calculations as well. The synthesized derivatives have Caco-2 permeability in the range of 1.07 to 1.38. A logBB > 0.3 for a small molecule would readily cross the blood brain barrier (BBB), while molecules with logBB < -1 are considered to penetrate the BBB poorly. All the synthesized compounds are in the range of -0.17 to -0.50 predicting them to be poorly penetrable through the BBB. These compounds are predicted to be metabolized by CYP3A4, similar to rivaroxaban which is metabolized by CYP3A4 (18 %) and CYP2J2 (14 %). The total clearance for the synthesized compounds was in the range of 0.23 to 0.58 ml/min/kg. Thus, the predicted physicochemical and pharmacokinetic properties of the newly synthesized compounds suggested their drug like behavior.

Table 4.7: Calculated physicochemical and pharmacokinetic properties of the synthesized compounds and rivaroxaban

Compound	Mol. Wt	No. of HBD	No. of HBA	Log P	PSA	No. of rotatable bonds	Caco2 permeability (log Papp in 10^{-6} cm/s)	% Intestinal Absorption (human)	BBB permeability (log BB)	Metabolism by	Total clearance log (ml/min/kg)
Rivaroxaban	435.88	1	6	2.51	175.48	5	1.28 (8.9 x 0.1 10^{-6} cm/s at 0.92 μM) *	92.80 (> 80%) #	-1.02	CYP3A4 CYP2J2@	0.29
187	497.95	1	5	4.71	207.54	6	1.20	93.52	-0.32	CYP3A4	0.25
201	474.10	1	5	4.76	193.71	5	1.07	91.49	-0.17	CYP3A4	0.23
203	500.02	1	6	4.94	207.38	6	1.28	92.61	-0.30	CYP3A4	0.58
208	503.98	1	6	4.77	205.18	6	1.17	92.14	-0.50	CYP3A4	0.49
224	494.97	1	6	4.27	208.96	6	1.38	94.97	-0.43	CYP3A4	0.51

HBD = Hydrogen Bond Donor; HBA = Hydrogen Bond Acceptor; PSA = Polar Surface Area; BBB = Blood Brain Barrier * Caco2 permeability (log Papp in 10^{-6} cm/s) of Rivaroxaban[29], # % Intestinal Absorption (human), @ Metabolic enzyme in human.

4.2 Furanopyrimidinone-based Thrombin inhibitors

The synthesis, biological activity, molecular docking studies and physicochemical properties of Furanopyrimidinone-containing derivatives are described under the following sub-heads:

4.2.1 Synthesis of Furanopyrimidinone-amide derivatives

4.2.2 Biological evaluation of the target compounds

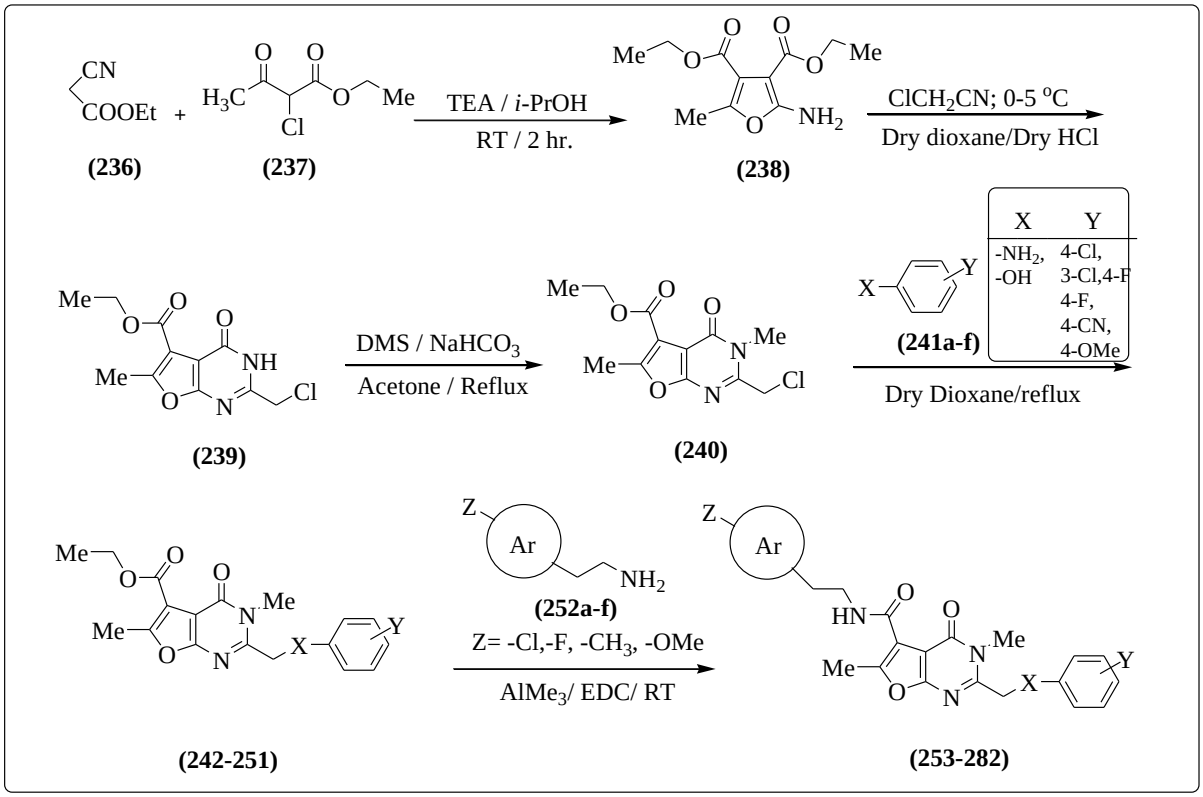
4.2.3 Molecular docking studies

4.2.4 Prediction of physicochemical properties

4.2.1 Synthesis of Furanopyrimidinone-amide derivatives

The synthetic route followed for the preparation of furanopyrimidinone amide target compounds is depicted in general scheme **4.7**. The first compound, 2-amino-5-methylfuran diethyl dicarboxylate (**238**) was synthesized from ethyl cyanoacetate (**236**) and ethyl 2-

chlroacetoacetate (**237**). Compound (**238**) when treated with chloroacetonitrile and dry HCl in dry dioxane resulted in the cyclized product, ethyl-2-(chloromethyl)-3,4-dihydro-6-methyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**239**) which after methylation using dimethyl sulphate afforded ethyl 2-((4-chloromethyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**240**). Compound **(240**) by nucleophilic substitution with anilines or phenols (**241a-f**) yielded compounds (**242-251**). Finally, the target compounds (**253-283**) were prepared from treatment of compounds (**242-251)** with substituted phenethylamines (**252a-f**) by Weinreb transamidation using trimethyl aluminium as the coupling reagent.



Scheme 4.7

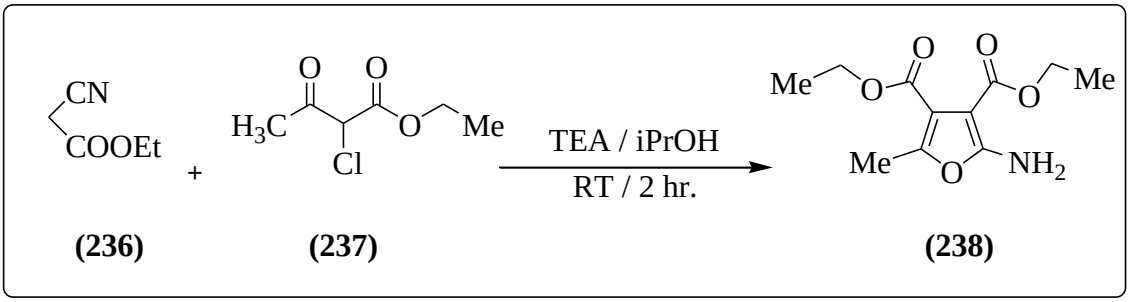
The research work planned for the synthesis of different intermediates (**238-251**) and the target compounds 4-oxo-*N*-phenethylfuro[2,3-*d*]pyrimidine-5-carboxamides (**253-282**) are discussed under following sub-headings,

1. Synthesis of diethyl 2-amino-5-methylfuran-3,4-dicarboxylate (**238**)

2. Synthesis of Ethyl 2-(chloromethyl)-3,4-dihydro-6-methyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**239**)
3. Synthesis of Ethyl 2-(chloromethyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**240**)
4. Synthesis of Ethyl 2-((arylamino)methyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate derivatives (**242-251**)
5. Synthesis of 2-((arylamino)methyl)-3,4-dihydro-3,6-dimethyl-4-oxo-*N*-phenethylfuro[2,3-*d*]pyrimidine-5-carboxamide derivatives (**253-282**).

4.2.1.1 Synthesis of diethyl 2-amino-5-methylfuran-3,4-dicarboxylate (**238**).

Ethyl cyanoacetate (**236**) and triethylamine together in isopropyl alcohol on treatment with ethyl-2-chloroacetoacetate (**237**) afforded diethyl 2-amino-5-methylfuran-3,4-dicarboxylate (**238**) through cyclization[12] (**scheme 4.8**). This compound exhibited IR peaks for -NH stretching in primary amino group at 3429 and 3325 cm^{-1} and for carbonyl (C=O stretching) of ester at 1708 cm^{-1} .



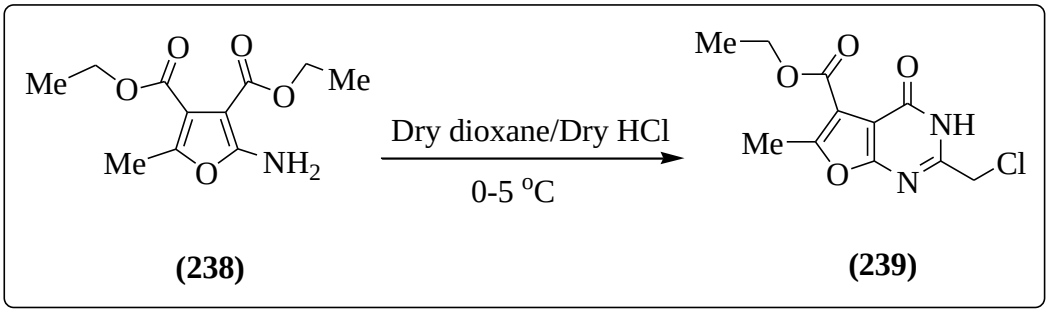
(236) **(237)** **(238)**

Scheme 4.8

4.2.1.2 Synthesis of ethyl 2-(chloromethyl)-3,4-dihydro-6-methyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**239**)

Diethyl 2-amino-5-methylfuran-3,4-dicarboxylate (**238**) was further cyclized into ethyl 2-(chloromethyl)-3,4-dihydro-6-methyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**239**) by the action of anhydrous chloroacetonitrile and dry HCl in dry dioxane solution (**scheme 4.9**). The IR spectrum displayed -NH stretching peak at 3430 cm^{-1} along with C=O stretching of carboxylate moiety at 1719 cm^{-1} and characteristic amide carbonyl (C=O) at 1676 cm^{-1}. Proton NMR of the compound offered signal at δ 12.24 (bs, 1H, N*H*) for amide proton and δ 4.53 (s, 2H, -CH_2Cl) singlet for methylene protons. A quartet for ester methylene at δ 4.32-4.28 (q, *J* = 7.0 Hz, 2H,

C*H*$_2$CH$_3$), a singlet for 6-methyl at δ 2.63 (s, 3H, Ar-C*H*$_3$) and triplet of ester methyl at δ 1.34 (t, *J* = 7.0 Hz, 3H, CH$_2$C*H*$_3$) were also observed. Mass spectrum furnished quasimolecular ion peak at *m/z* 271 $[M+1]^+$ and isotopic peak at 272 $[M+2]^+$.

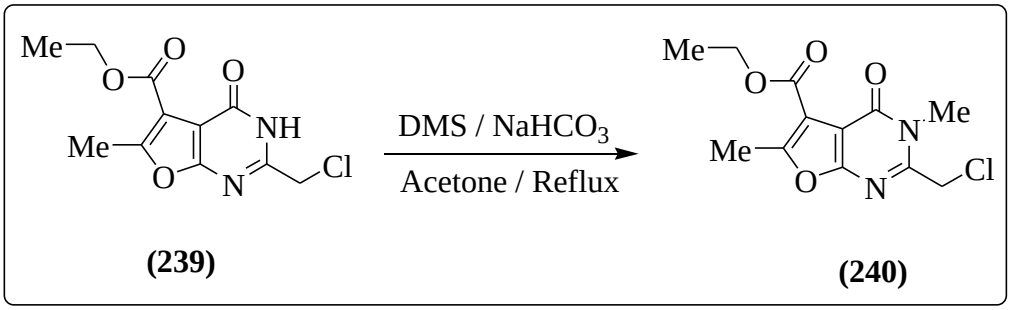


(238) **(239)**

Scheme 4.9

4.2.1.3 Synthesis of ethyl 2-chloromethyl-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**240**)

Methylation of the intermediate (**239**) using dimethyl sulphate under inert nitrogen conditions provided ethyl 2-(chloromethyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**240**) (**scheme 4.10**) which exhibited absence of -NH stretching and presence of peaks at 1727 (ester C=O) and 1689 (amide C=O) cm^{-1}. ^{1}H-NMR revealed singlet at δ 4.59 (s, 2H, -C*H*$_2$Cl) of chloromethylene and δ 4.40 (q, *J* = 7.1 Hz, 2H, C*H*$_2$CH$_3$) quartet for ester methylene. A singlet for N-methyl at δ 3.72 (s, 3H, -NC*H*$_3$) confirmed methylation of amide -NH proton. δ 2.66 (s, 3H, Fur-C*H*$_3$) singlet and 1.41 (t, *J* = 7.2 Hz, 3H, CH$_2$C*H*$_3$) triplet peaks were also seen in the aliphatic region. Mass peaks were recorded at m/z 284 $[M]^+$ and 286 $[M+2]^+$

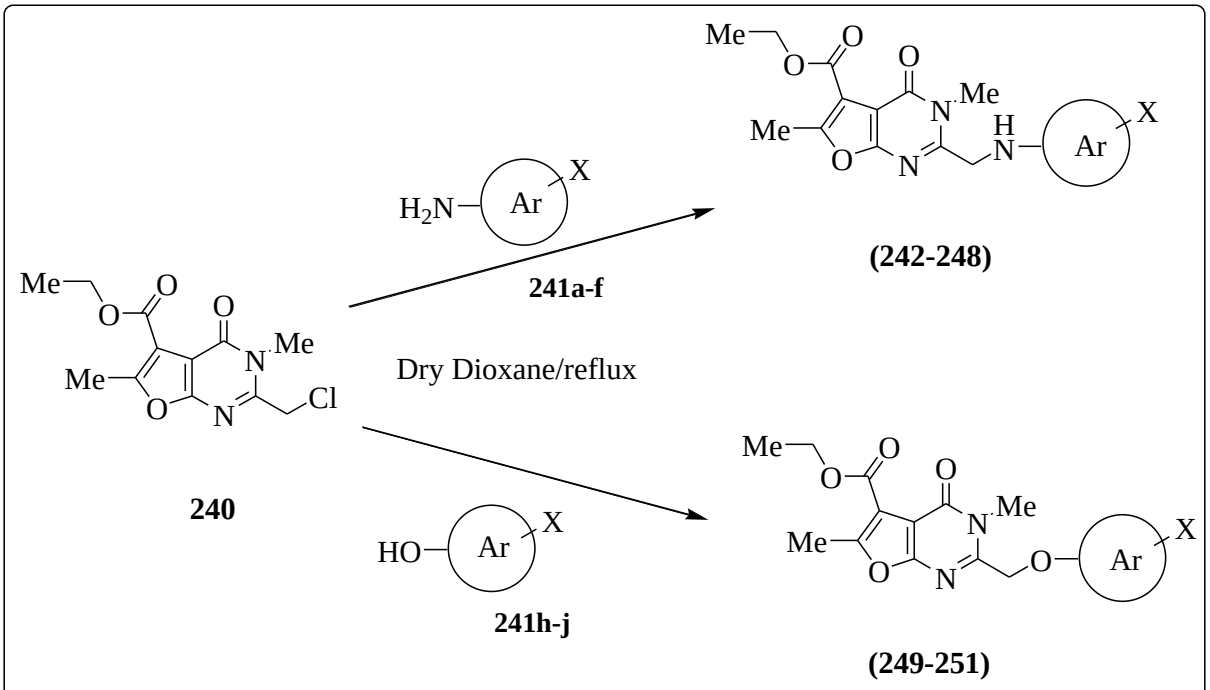


(239) **(240)**

Scheme 4.10

4.2.1.4 Synthesis of ethyl 2-((arylamino)methyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate derivatives (242-251)

A nucleophilic substitution of ethyl 2-chloromethyl-1,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**240**) with substituted anilines and phenols (**241a-f**) in dry dioxane solvent under reflux conditions provided ethyl 2-(phenylamino)methyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate derivatives (**242-251**) (scheme **4.11**).

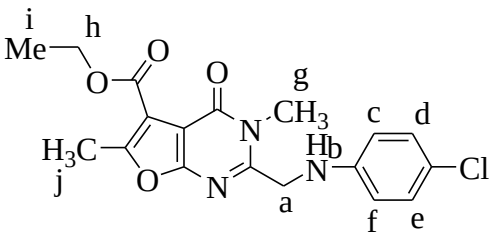


Compound	Ar-X	Compound	Ar-X
242	4-ClPh	**247**	4-OMePh
243	4-FPh	**248**	5-Cl-pyrid-2-yl
244	3-Cl, 4-FPh	**249**	4-ClPh
245	4-CNPh	**250**	4-FPh
246	4-EtPh	**251**	4-CNPh

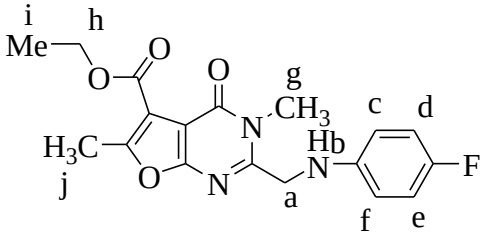
Scheme 4.11

Infrared spectrum of compound (**242**) revealed bands of -NH stretching at 3377 cm^{-1}, ester carbonyl at 1732 cm^{-1} and amide carbonyl at 1683 cm^{-1}. Proton magnetic resonance of the compound offered signals for aromatic protons as two separate doublets because of *ortho*

coupling in phenyl ring, at δ 7.19-7.17 (d, *J* = 8.75 Hz, 2H*d, e*) and 6.68-6.66 (d, *J* = 8.75 Hz, 2H*c, f*). A singlet for secondary amino proton at δ 5.11 (s, 1H*b*, -N*H*) and a quartet for two methylene (CH_2CH_3) protons at δ 4.41-4.39 (q, *J* = 7.0 Hz, 2H*h*) were also observed. Three different singlets appeared at δ 4.33 (s, 2H*a*, CH_2NH) for methylene protons, δ 3.63 (s, 3H*g*, -NCH_3) for tertiary *N*-methyl and δ 2.68 (s, 3H*j*, Fur-CH_3) for methyl protons of furan ring. A triplet for methyl group of the ester moiety appeared at δ 1.43 (t, *J* = 7.5 Hz, 3H*i*, CH_2CH_3). The mass spectrum of the compound showed m/z 376 $[M+1]^+$ and an isotopic peak at 377 $[M+2]^+$.



(**242)**

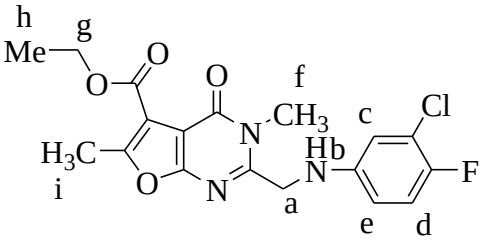


(243)

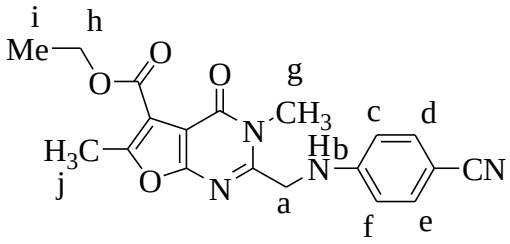
Compound (**243**) in its IR spectrum showed peaks at 3371 cm^{-1} for -NH stretching, 1734 cm^{-1} for ester C=O stretching and 1681 cm^{-1} for amide C=O stretching. In its PMR, two different multiplets representing two aromatic protons each observed at δ 6.98-6.95 (m, 2H*d, e*, Ar-*H*) and 6.72-6.69 (m, 2H*c, f*, Ar-*H*) as. A singlet of amino proton at δ 4.92 (s, 1H*b*, -N*H*), a quartet at δ 4.45-4.44 (q, *J* = 7.0 Hz, 2H*h*, CH_2CH_3) and a singlet at δ 4.36 (s, 2H*a*, CH_2NH) for different methylene protons were also seen. Separate singlet peaks at δ 3.66 (s, 3H*g*, -NCH_3) for N-methyl and δ 2.69 (s, 3H*j*, Fur-CH_3) for methyl protons of furan were also present. A triplet peak for carboxylate methyl protons displayed at δ 1.44 (t, *J* = 7.0 Hz, 3H*i*, CH_2CH_3). Carbon NMR of the compound exhibited signals at δ 162.66, 161.82, 158.22, 157.33, 155.43, 143.13, 116.03, 115.86, 114.26, 114.20, 111.07, 103.36 and at δ 61.10, 47.05, 41.01, 29.53, 14.32, 13.93 for aliphatic carbons. ESI-MS of compound displayed a peak at *m/z* 360 $[M+1]^+$.

Compound (**244**) displayed peaks for NH stretching at 3389 cm^{-1}, for ester C=O stretching at 1722 cm^{-1} and amide C=O stretching at 1678 cm^{-1} in its FT-IR spectrum. PMR spectrum of the compound displayed a multipet at δ 7.07-6.98 (m, 1H*d*, Ar-H) and two doublets for each single aromatic proton at δ 6.75 (d, 1H*c*, Ar-*H*) and 6.62 (d, 1H*e*, Ar-*H*). Individual peaks were observed at δ 5.07 (s, 1H*b*, N*H*) as singlet, 4.44 (q, 2H*g*, CH_2CH_3) as quartet, 4.34

(d, 2H*a*, C*H*$_2$NH) doublet along with signals at δ 3.65 (s, 3H*f*, N-C*H*$_3$) singlet, 2.69 (s, 3H*i*, Fur-C*H*$_3$) singlet and 1.44 (t, 3H*h*, CH$_2$C*H*$_3$) triplet for various methyl protons in the aliphatic region. The mass spectrum revealed peaks at *m/z* 394 $[M+1]^+$ and 395 $[M+2]^+$.



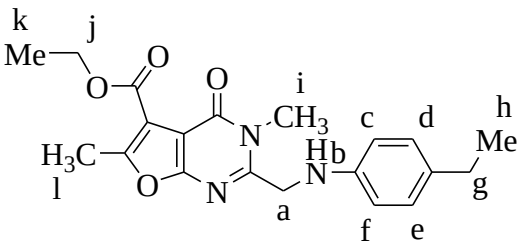
(244)



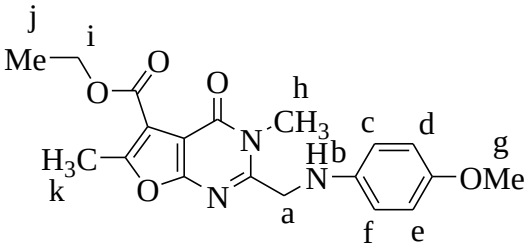
(245)

In the IR spectrum compound (**245**) offered -NH stretching at 3366 cm^{-1}, characteristic -CN stretching at 2212 cm^{-1}, ester C=O stretching at 1724 cm^{-1} and amide C=O stretching at 1680 cm^{-1}. Its proton NMR revealed two individual doublets each representing two aromatic protons at δ 7.51-7.49 (d, *J* = 8.6 Hz, 2H*d, e*) and 6.73-6.71 (d, *J* = 8.6 Hz, 2H*c, f*). Aliphatic region showed signals at δ 5.70 (s, 1H*b*, NH) as singlet for secondary amino proton along with δ 4.44-4.38 (m, 4H*a, h*, C*H*$_2$CH$_3$ & C*H*$_2$NH) as multiplet for four methylene protons and three separate signals representing different types of methyl protons at 3.64 (s, 3H*g*, N-C*H*$_3$), 2.68 (s, 3H*j*, Fur-C*H*$_3$) and 1.42 (t, *J* = 7.2 Hz, 3H*i*, CH$_2$C*H*$_3$). ESI-MS of compound offered $[M+1]^+$ peak at m/z 367.

Compound (**246**) in its IR spectrum revealed signals at 3398 cm^{-1} of NH stretching, 1730 cm^{-1} of ester C=O stretching and 1690 cm^{-1} of amide C=O stretching. ^{1}H-NMR spectrum of the compound displayed two separate doublets at δ 7.11-7.07 (d, *J* = 8.0 Hz, 2H*c, f*) and 6.73-6.69 (d, *J* = 8.0 Hz, 2H*d, e*) for aromatic protons. Individual peaks were noticed at δ 4.88 (s, 1Hb, -N*H*) as a singlet for single proton, δ 4.48-4.37 (m, 4H*a, j*, -C*H*$_2$NH & -C*H*$_2$CH$_3$) as multiplet for four methylene protons, δ 3.66 (s, 3H*i*, N-C*H*$_3$) singlet for three protons of methyl group and δ 2.69 (s, 3H*l*, Fur-C*H*$_3$) singlet for other methyl protons. A quartet for the methylene protons at δ 2.63-2.59 (q, 2H*g*, Ar-C*H*$_2$CH$_3$) and two different triplets each for two methylene protons also appeared at δ 1.44 (t, 3H*k*, CH$_2$C*H*$_3$) and δ 1.21 (t, 3H*h*, Ar-CH$_2$C*H*$_3$) respectively. Peaks appeared at m/z 370 $[M+1]^+$ and 371 $[M+2]^+$ in its mass spectrum.



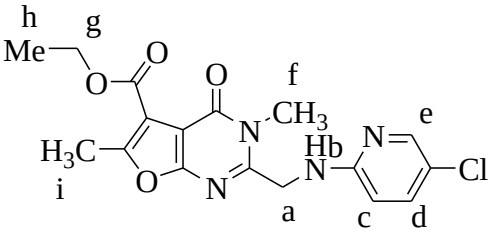
(246)



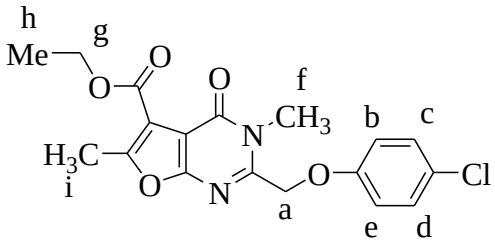
(247)

IR spectrum of compound (**247**) exhibited bands at 3401 cm^{-1} for NH stretching and 1725 cm^{-1} for C=O stretching of ester. In its PMR spectrum, compound (**247**) showed signals at δ 6.86-6.84 (d, *J* = 8.8 Hz, 2H*c*, *f*, Ar-*H*) and 6.76-6.73 (d, *J* = 8.8 Hz, 2H*d*, *e*, Ar-*H*) as two doublets each for two aromatic protons. A quartet for two protons at δ 4.46-4.44 (q, *J* = 7.2 Hz, 2H*i*, CH_2CH_3) and a singlet at δ 4.38 (s, 2H*a*, CH_2NH) for two methylene protons followed by three separate singlets at δ 3.78 (s, 3H*g*, -OCH_3), 3.67 (s, 3H*h*, N-CH_3) and 2.70 (s, 3H*k*, Fur-CH_3) corresponding to three protons each and a triplet at δ 1.45 (t, *J* = 7.2 Hz, 3H*j*, -CH_2CH_3) representing three aliphatic protons were also observed.

Peaks were observed at 3413 cm^{-1} for NH stretching, 1732 cm^{-1} for C=O stretching and 1703 cm^{-1} for C=O stretching in the IR spectrum of compound (**248**). ^{1}H-NMR spectrum of the compound showed multiplet for one proton at δ 8.08-8.07 (d, *J* =2.5 Hz, 1H*e*) and a doublet of doublet due to *meta* coupling of H*d* with H*e* proton and *ortho* coupling with H*c* proton at δ 7.44-7.42 (dd, *J* = 2.5, 7.5 Hz, 1H*d*). Additional signals as doublet at δ 6.63-6.61 (d, 1H*c*, Ar-*H*), a singlet at 5.73 (s, 1H*b*, -CH_2NH), a doublet at δ 4.68 (d, *J* = 4.5 Hz, 2H*a*, CH_2NH), a quartet at δ 4.44-4.42 (q, *J* = 7.0 Hz, 2H*g*, CH_2CH_3), two singlets at δ 3.68 (s, 3H*f*, NCH_3) and 2.68 (s, 3H*i*, Fur-CH_3) along with a triplet at δ 1.45 (t, *J* = 7.5 Hz, 3H*h*, CH_2CH_3) were also seen. Mass peaks were observed at *m/z* 377 $[M+1]^+$ and 378 $[M+2]^+$.



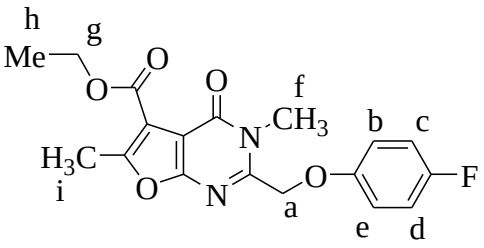
(248)



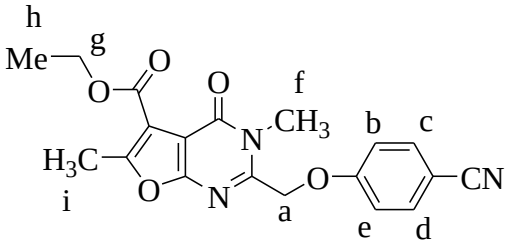
(249)

Compound (**249**) showed characteristic IR peaks for C=O stretching of carboxylate at 1738 cm^{-1} and C=O stretching of amide at 1695 cm^{-1}. In its proton NMR displayed peaks at δ 7.27-7.25 (d, *J* = 8.4 Hz, 2H*c*, *d*) and 6.97-6.95 (d, *J* = 8.8 Hz, 2H*b*, *e*) as two doublets in aromatic region. A quartet for two methylene protons at 4.43-4.42 (q, *J* = 7.2 Hz, 2H*g*, -C*H*$_2$CH$_3$), two individual singlets at 3.72 (s, 3H*f*, -NC*H*$_3$) and 2.68 (s, 3H*i*, Fur-C*H*$_3$) for methyl protons and a triplet at δ 1.42 (t, *J* = 6.8 Hz, 3H*h*, -CH$_2$C*H*$_3$) were also recorded.

The IR spectrum of compound (**250**) afforded peaks of ester C=O stretching at 1739 cm^{-1} and amide C=O stretching at 1701 cm^{-1}. The proton resonance displayed signals of a broad multiplet for four aromatic protons at δ 6.99-6.96 (m, 4H*b*, *c*, *d*, *e*), a singlet at δ 5.12 (s, 2H*a*, C*H*$_2$-O), a quartet at δ 4.43-4.41 (q, *J* = 6.8 Hz, 2H*g*, C*H*$_2$CH$_3$), two separate singlets for N-methyl and furan-methyl at δ 3.73 (s, 3H*f*, -NC*H*$_3$) and 2.67 (s, 3H*i*, Fur-C*H*$_3$) respectively, and a triplet for methylene at δ 1.42 (t, *J* =7.2 Hz, 3H*h*, CH$_2$C*H*$_3$).



(250)



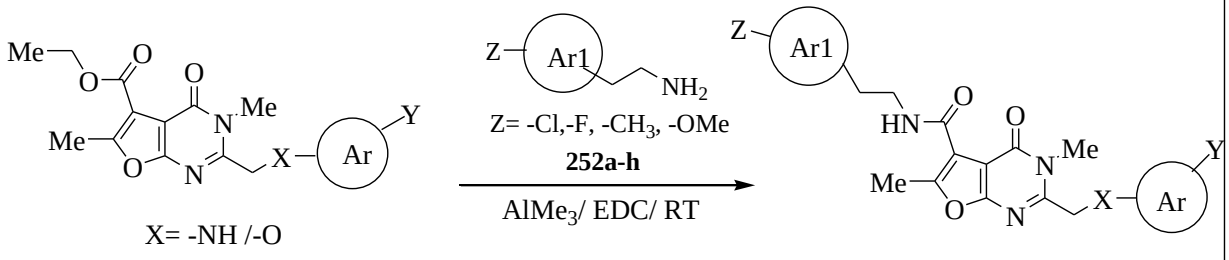
(251)

Compound (**251**) exhibited characteristic IR peaks for CN stretching at 2221 cm^{-1} along with ester C=O stretching at 1723 cm^{-1} and amide C=O stretching at 1686 cm^{-1}. ^{1}H-NMR spectrum of the compound revealed peaks at δ 7.64-7.62 (d, *J* = 7.0 Hz, 2H*c*, *d*) and 7.14-7.12 (d,

J = 7.0 Hz, 2H*b, e*) as two doublets for aromatic protons. A singlet for two methylene protons at δ 5.24 (s, 2H*a*, C*H*$_2$-O) along with a quartet at 4.44-4.42 (q, *J* = 7.2 Hz, 2H*g*, C*H*$_2$CH$_3$), methyl-protons singlets at δ 3.73 (s, 3H*f*, NC*H*$_3$) and 2.69 (s, 3H*i*, Fur-C*H*$_3$), followed by a triplet at δ 1.42 (t, *J* = 7.2 Hz, 3H*h*, CH$_2$C*H*$_3$) were also seen.

4.2.1.5 Synthesis of 2-((arylamino)methyl)-3,4-dihydro-3,6-dimethyl-4-oxo-*N*-phenethylfuro[2,3-*d*]pyrimidine-5-carboxamide derivatives (253-282).

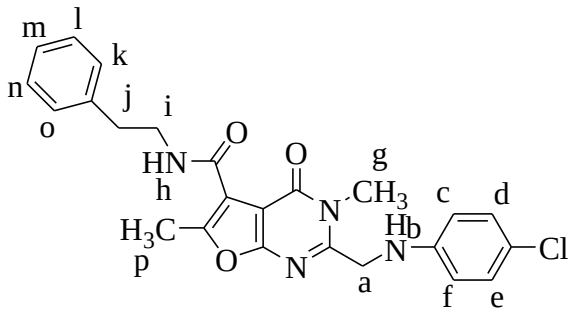
Finally ethyl 2-((3/4-substituted phenylamino)methyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-d]pyrimidine-5-carboxylate derivatives (**242-251**) were derivatized into targeted carboxamide compounds (**253-282**) by Weinreb transamidation[13] method using different phenethylamines (**252a-f**) and trimethyl aluminium as ester-amine coupling reagent (**scheme 4.12**). Absence of ester C=O stretching at ≈1730 cm^{-1} and presence of amide C=O stretching at ≈1660 cm^{-1} confirmed the formation of the target compounds. Compound (**253**) in its IR spectra exhibited characteristic amide C=O stretching at 1665 (HNC=O) and 1639 cm^{-1} (Me-N-C=O). Proton magnetic resonance (PMR) spectrum of compound (**253**) revealed signals at δ 10.03 (t, *J* = 5.0 Hz, 1H*h*) as triplet for amide (N*H*CO) proton and two multiplets for five aromatic protons at δ 7.33-7.28 (m, 5H*k, l, m, n, o,* Ar-*H*). Other aromatic protons displayed a multiplet at δ 7.24-7.21 (m, 2H*d, e,* Ar-*H*) and a doublet for two protons at 6.71-6.69 (d, *J* = 8.0 Hz, 2H*c, f,* Ar-*H*). Aliphatic region exhibited signals at δ 4.40 (s, 2H*a*) as singlet for two methylene (C*H*$_2$-NH) protons followed by a multiplet corresponding to five protons of amide methyl and methylene linker at δ 3.70-3.67 (m, 5H*g, i,* CH$_2$-C*H*$_2$-NH & N-C*H*$_3$). A triplet for two other methylene protons at δ 2.98 (t, *J* = 8.0 Hz, 2H*j*, C*H*$_2$-CH$_2$-NH) along with a singlet for methyl protons at δ 2.85 (s, 3H*p*, Furan-C*H*$_3$) were also observed. A mass spectrum of the compound offered a peak at *m/z* 451.3 [M+H]$^+$.



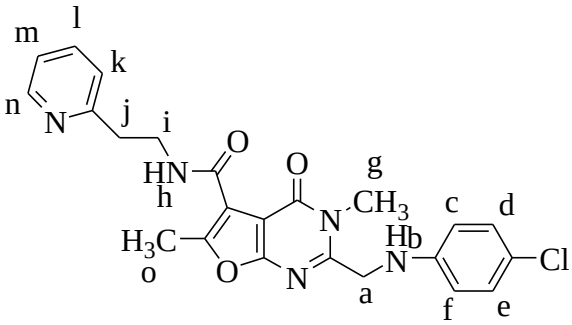
(242-251) **(253-282)**

Compound	Ar	Ar1	Compound	Ar	Ar1
253	4-ClPh	Ph	**268**	4-CNPh	4-MePh
254	4-ClPh	2-Pyridyl	**269**	4-CNPh	4-FPh
255	4-ClPh	4-FPh	**270**	4-CNPh	4-ClPh
256	4-ClPh	4-OMePh	**271**	4-CNPh	4-OMePh
257	4-ClPh	4-MePh	**272**	4-CNPh (O)	Ph
258	4-FPh	Ph	**273**	4-CN Ph (O)	4-FPh
259	4-FPh	4-ClPh	**274**	4-CN Ph (O)	4-ClPh
260	4-FPh	4-OMePh	**275**	4-CNPh (O)	2-Pyridyl
261	4-FPh	4-MePh	**276**	4-CNPh (O)	4-MePh
262	3-Cl, 4-FPh	Ph	**277**	4-ClPh (O)	Ph
263	3-Cl, 4-FPh	4-ClPh	**278**	4-ClPh (O)	4-FPh
264	3-Cl, 4-FPh	4-FPh	**279**	4-F Ph (O)	Ph
265	3-Cl, 4-FPh	4-OMePh	**280**	4-F Ph (O)	4-ClPh
266	3-Cl, 4-FPh	4-MePh	**281**	4-OMe	4-FPh
267	4-CNPh	Ph	**282**	5-Cl (Pryd)	4-MePh

Scheme 4.12



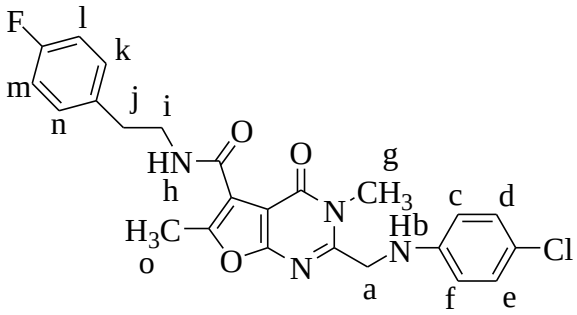
(253)



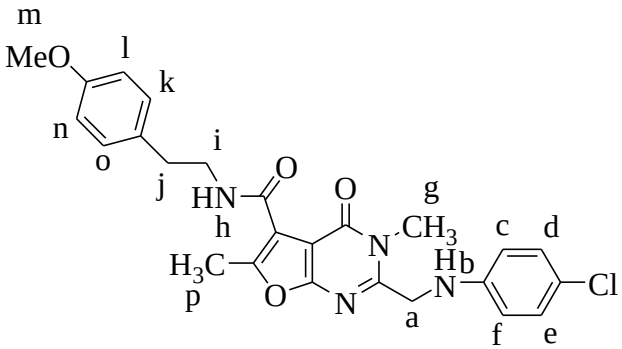
(254)

Compound (**254**) displayed infrared bands for NH stretching at 3390 and 3257 cm^{-1} with amide C=O stretching at 1668 cm^{-1}. PMR spectrum of the compound revealed a triplet at δ 10.15 (t, *J* = 5.4 Hz, 1H*h*, CON*H*) of amide proton, a multiplet for a proton at δ 8.51-8.50 (m, 1H*n*, Ar-*H*) and two different doublets at δ 7.72-7.70 (d, *J* = 7.7 Hz, 1H*l*, Ar-*H*) and 7.31-7.29 (d, *J* = 7.7 Hz, 1H*k*, Ar-*H*) for one proton each and a multiplet at δ 7.24-7.22 (m, 1H*m*, Ar-*H*). Two multiplets for four aromatic protons also appeared at δ 7.11-7.09 (m, 2H*d, e*, Ar-*H*) and 6.74-6.72 (m, 2H*c, f*, Ar-*H*). A triplet at δ 6.38 (t, *J* = 6.0 Hz, 1H*b*, N-*H*), a doublet for methylene at δ 4.51-4.50 (d, *J* = 5.9 Hz, 2H*a*, C*H*$_2$-NH), a quartet for two methylene protons at δ 3.69-3.67 (q, *J* = 7.0 Hz, 2H*i*, CH$_2$-C*H*$_2$-NH) and a singlet of methyl protons at δ 3.61 (s, 3H*g*, N-C*H*$_3$) were observed additionally with a triplet at δ 2.99 (t, *J* = 7.3 Hz, 2H*j*, C*H*$_2$-CH$_2$) and a singlet at δ 2.68 (s, 3H*o*, Ar-C*H*$_3$). The HR-MS spectrum of the compound exhibited m/z 452.1484 $[M+H]^+$.

IR spectrum of compound (**255**) exhibited NH stretching bands at 3375 and 3239 cm^{-1} and amide C=O stretching at 1659 cm^{-1}. ^{1}H-NMR of the compound revealed a triplet for amide proton at δ 10.03 (t, *J* = 7.5 Hz, 1H*h*, CON*H*). A multiplet for four protons at δ 7.28-7.21 (m, 4H*k, l, m, n*), two multiplets for four protons at δ 6.99-6.96 (m, 2H*d, e*) and 6.73-6.70 (m, 2H*c, f*) appeared in the aromatic region. A singlet for two methylene protons at δ 4.40 (s, 2H*a*, C*H*$_2$-NH), a multiplet for five protons of methyl and methylene at δ 3.69-3.64 (m, 5H*g, i*, -N-C*H*$_3$ & -CH$_2$-C*H*$_2$-NH), a triplet at δ 2.95 (t, *J* = 7.5 Hz, 2H*j*, C*H*$_2$-CH$_2$) and singlet for methyl from furan ring at δ 2.84 (s, 3H*o*, Fur-C*H*$_3$) were observed in aliphatic area. The mass spectrum of the compound was noticed at *m/z* 469.3 $[M+H]^+$.



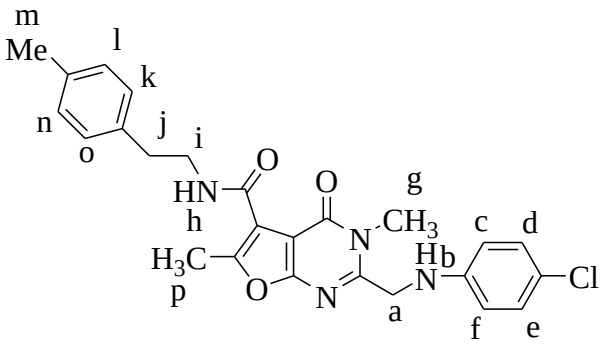
(**255**)



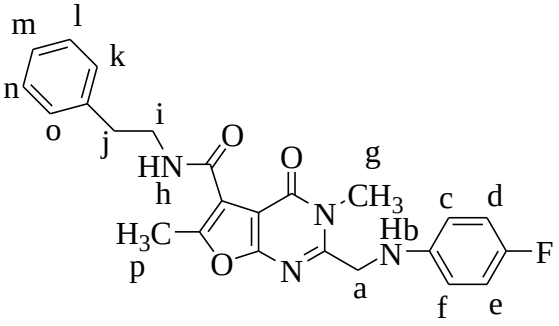
(256)

Compound (**256**) showed peaks at 3396 and 3250 cm^{-1} for NH stretching and amide C=O at 1674 cm^{-1} in its infrared spectrum. The PMR spectrum of compound gave peaks at δ 9.97 (t, *J* = 5.6 Hz, 1H*h*, CON*H*) a triplet for amide proton and a doublet for four protons at δ 7.20-7.18 (d, *J* = 8.0 Hz, 4H*d, e, k, o*) along with two doublets at δ 6.84-6.82 (d, *J* = 8.0 Hz, 2H*c, f*) and 6.68-6.66 (d, *J* = 8.4 Hz, 2H*l, n*) for two protons each. One singlet for methylene protons at δ 4.38 (s, 2H*a*, C*H*$_2$-NH) and another at δ 3.78 (s, 3H*m*, OC*H*$_3$) appeared for methoxy protons. A multiplet accounting for five protons at δ 3.67-3.60 (m, 5H*g, i*, CH_2-C*H*$_2$-NH & -N-C*H*$_3$), a triplet for methylene protons at δ 2.89 (t, *J* = 8.4 Hz, 2H*j*, C*H*$_2$-CH_2) and a singlet for methyl protons at δ 2.82 (s, 3H*p*) too were noticed.

The IR spectrum of the compound (**257**) exhibited NH stretching at 3250 cm^{-1} and amide C=O stretching at 1672 cm^{-1} and 1639 cm^{-1}. The PMR of the compound displayed a triplet at δ 10.02 (t, *J* = 5.5 Hz, 1H*h*, CON*H*) and a multiplet corresponding to four protons at δ 7.23-7.19 (m, 4H*d, e, k, o*). Two doublets for four aromatic protons were noticed at δ 7.13-7.12 (d, *J* = 7.5 Hz, 2H*l, n*) and 6.72-6.71 (d, *J* = 8.5 Hz, 2H*c, f*). Aliphatic protons were observed at δ 4.41 (d, *J* = 4.5 Hz, 2H*a*, C*H*$_2$-NH) as doublet, δ 3.70 (s, 3H*g*, N-C*H*$_3$) as a singlet and 3.68-3.67 (q, *J* = 7.5 Hz, 2H*i*, CH_2-C*H*$_2$-NH) as a quartet. A triplet for two methylenes at δ 2.95 (t, *J* = 7.5 Hz, 2H*j*, C*H*$_2$-CH_2) and two singlets for six methyl protons also appeared at δ 2.85 (s, 3H*p*, O-C-C*H*$_3$) and 2.34 (s, 3H*m*). The mass spectrum of the compound offered a peak at *m/z* 465.4 for $[M+H]^+$.

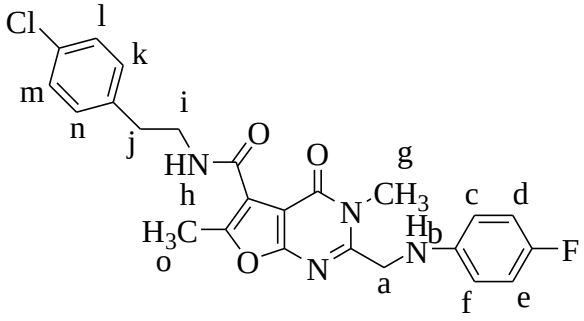


(**257**)

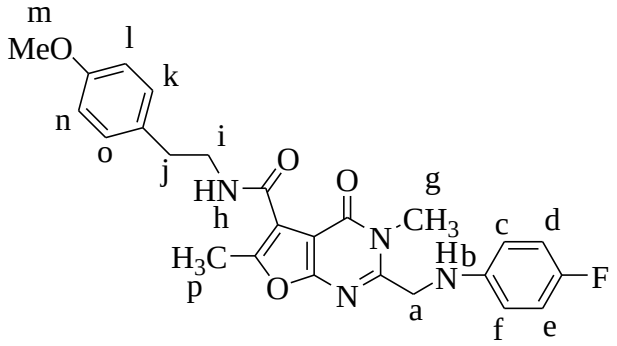


(258)

Compound (**258**) in its IR spectrum showed peaks at 3310 and 3044 cm^{-1} for NH stretching and for amide C=O stretching at 1668 cm^{-1}. The proton NMR of the compound afforded a signal at δ 10.02 (t, *J* = 5.2 Hz, 1H*h*, CON*H*) as triplet for proton of amide and a multiplet contributing four aromatic protons at δ 7.31-7.27 (m, 4H*k*, *l*, *n*, *o*). Other aromatic protons appeared as three different multiplets at δ 7.23-7.18 (m, 1H*m*), 6.96-6.91 (m, 2H*d*, *e*) and 6.70-6.67 (m, 2H*c*, *f*). A singlet for two protons at δ 4.36 (s, 2H*a*, CH_2-NH) and a multiplet for five protons at δ 3.68-3.62 (m, 5H*g*, *i*) were noticed with a triplet for two methylenes at δ 2.95 (t, *J* = 7.9 Hz, 2H*j*, CH_2-CH_2) and singlet for three methyl protons at δ 2.81 (s, 3H*p*, O-C-CH_3). ^{13}C-NMR of the compound revealed carbon signals at δ 162.12, 161.27, 160.32, 158.71, 154.70, 143.0, 139.31, 128.81, 128.44, 126.25, 116.09, 115.91, 114.34, 114.28, 112.44, 102.92, and at δ 47.17, 41.01, 35.89, 29.84 and 14.04. Mass spectrum of the compound offered peak for $[M+H]^+$ at m/z 435.3.



(**259**)



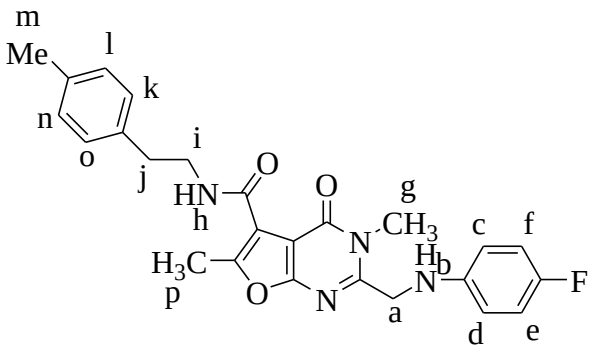
(260)

Compound (**259**) revealed NH stretching at 3390 and 3253 cm^{-1} along with amide C=O at 1677 and 1648 cm^{-1} in the IR spectrum. PMR spectrum of the compound offered triplet for amide proton at δ 9.99 (t, *J* = 5.2 Hz, 1H*h*, CON*H*) and a multiplet representing four aromatic protons at δ 7.24-7.19 (m, 4H*k, l, m, n*). Other four protons revealed a doublet at δ 6.95 (d, *J* = 8.8 Hz, 2H*d, e*) and a multiplet at δ 6.71-6.67 (m, 2H*c, f*) in aromatic region. Three singlet peaks were observed at δ 4.85 (s, 1H*b*, N*H*) for amino proton, δ 4.38 (s, 2H*a*, CH_2-NH) for two methylenes attached to amino group and at δ 3.78 (s, 3H*g*, -N-CH_3) for methyl protons. A multiplet and triplet both for methylene linker exhibited signals at δ 3.67-3.61 (m, 2H*i*, -CH_2-CH_2-NH) and δ 2.90 (t, *J* = 7.2 Hz, 2H*j*, CH_2-CH_2) respectively with a singlet for methyl protons at δ 2.81 (s, 3H*o*). The mass spectrum of the compound was seen at m/z 469.3 $[M+H]^+$.

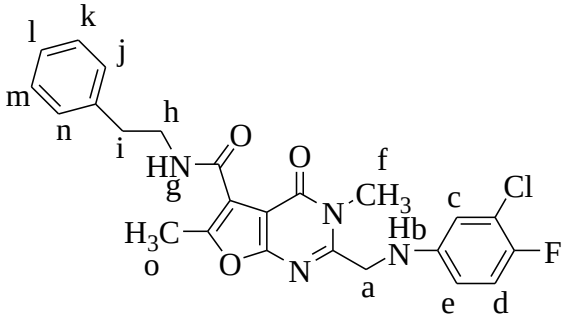
Compound (**260**) offered peaks at 3387 and 3258 cm^{-1} for NH stretching and 1673 cm^{-1} for amide C=O stretching in its IR spectrum. The PMR spectrum of the compound displayed signals at δ 9.98 (s, *J* = 5.2 Hz, 1H*h*, CON*H*) as amide proton triplet along with three doublets accounting for two protons each at δ 7.20-7.18 (d, *J* = 8.8 Hz, 2H*k, o*), 6.97-6.95 (d, *J* = 8.8 Hz, 2H*l, n*) and 6.84-6.82 (d, *J* = 8.8 Hz, 2H*d, e*) in addition to a multiplet for two protons at δ 6.71-6.68 (m, 2H*c, f*) in the aromatic region. Four singlets were observed at δ 4.85 (s, 1H*b*), 4.38 (s, 2Ha, CH_2-NH), 3.78 (s, 3H*g*) and 3.68 (s, 3H*m*). A multiplet at δ 3.63 (m, 2H*i*, CH_2-CH_2-NH) and a triplet at δ 2.90 (t, *J* = 8.0 Hz, 2H*j*, CH_2-CH_2) for four methylene protons were seen with a singlet for methyl protons at δ 2.82 (s, 3H*p*, O-C-CH_3). The HR-MS spectrum of the compound exhibited signal at m/z 465.1933 $[M+H]^+$.

The IR spectrum of the compound (**261**) displayed peaks at 1668 cm^{-1} for amide C=O stretching. PMR spectrum of the compound showed a triplet for amide proton at δ 10.03 (t, *J* = 5.6 Hz, 1H*h*, CON*H*) and two doublets for four protons at δ 7.21-7.19 (d, *J* = 7.8 Hz, 2H*k, o*) and 7.13-7.11 (d, *J* = 7.8 Hz, 2H*l, n*). Two multiplets were noticed at δ 7.00-6.95 (m, 2H*e, f*) and 6.73-6.70 (m, 2H*c, d*) for other aromatic protons. Aliphatic protons displayed singlet for two methylenes at δ 4.40 (s, 2H*a*, CH_2-NH) and for methyl of ring amide at δ 3.70 (s, 3H*g*, NCH_3) as well as a quartet at δ 3.69-3.67 (q, *J* = 7.6 Hz, 2H*i*, CH_2-CH_2-NH) and a triplet at δ 2.94 (t, *J* = 7.2 Hz, 2H*j*, CH_2-CH_2). Two singlets for two different methyl-protons also appeared at δ 2.84 (s,

3H*p*, O-C-C*H*$_3$) and 2.34 (s, 3H*m*). The mass spectrum of compound offered a peak at *m/z* 449.3 $[M+H]^+$



(**261)**

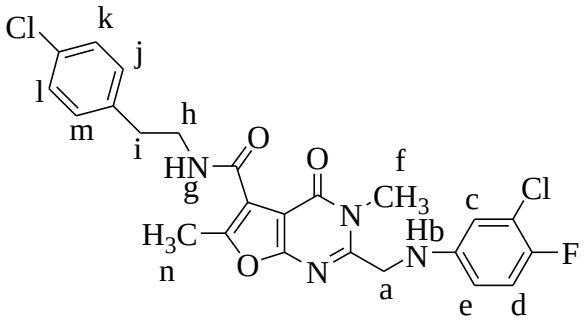


(262)

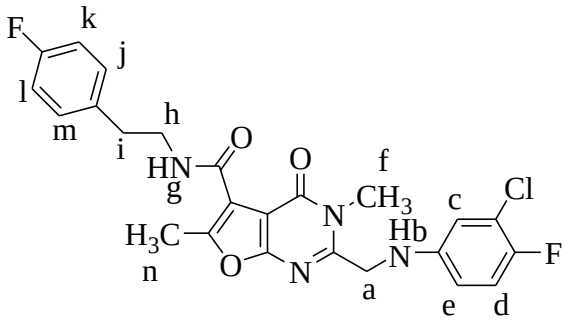
Compound (**262**) revealed NH stretching at 3384 and 3246 cm^{-1} along with amide C=O stretching at 1672 cm^{-1}. In its PMR spectrum compound (**262**) exhibited a triplet at δ 10.03 (t, *J* = 5.5 Hz, 1H*g*, CON*H*), two multiplets at δ 7.33-7.30 (m, 4H*j*, *k*, *m*, *n*) for four aromatic protons and δ 7.24-7.23 (m, 1H*l*) for a single proton. A triplet representing one proton at δ 7.04 (t, *J* = 9.0 Hz, 1H*d*) and two multiplets for two protons were also observed at δ 6.77-6.75 (m, 1H*c*) and 6.64-6.61 (m, 1H*e*). Among the aliphatic protons peaks were noticed at δ 5.05 (s, 1H*a*) as a singlet, δ 4.38 (d, *J* = 3.5 Hz, 2H*b*, C*H*$_2$-NH) as doublet and two multiplets at δ 3.70-3.68 (m, 5H*f*, *h*, -N-C*H*$_3$ &-CH$_2$-C*H*$_2$-NH) and 3.0-2.97 (m, 2H*i*, C*H*$_2$-CH$_2$) with a singlet at δ 2.85 (s, 3H*o*, O-C-C*H*$_3$).

In the IR spectrum of compound (**263**) peaks for NH stretching at 3394 and 3237 cm^{-1} and for C=O stretching at 1682 and 1641 cm^{-1} were observed respectively. The PMR spectrum of the compound exhibited a triplet for amide proton at δ 10.00 (t, *J* = 5.5 Hz, 1H*g*, CON*H*) and a multiplet corresponding to four aromatic protons at δ 7.28-7.22 (m, 4H*j*, *k*, *l*, *m*, Ar-*H*). A triplet for a proton at δ 7.04 (t, *J* = 9.0 Hz, 1H*d*) and two multiplets for two protons each at δ 6.77-6.75 (m, 1H*c*) and 6.64-6.61 (m, 1H*e*) were observed in aromatic region. Aliphatic protons showed two singlets at δ 5.04 (s, 1H*b*) and 4.38 (s, 2H*a*, C*H*$_2$-NH) including a multiplet for five protons at δ 3.69-3.65 (m, 5H*f*, *h*, N-C*H*$_3$ & CH$_2$-C*H*$_2$-NH). Two methylene protons as a triplet at δ 2.95 (t, *J* = 7.5 Hz, 2H*i*, C*H*$_2$-CH$_2$) and three methyl protons as a singlet at δ 2.84 (s, 3H*n*, O-C-C*H*$_3$)

also appeared. The mass spectrum of the compound displayed quasimolecular ion peak at *m/z* 503.2 $[M+H]^+$



(263)

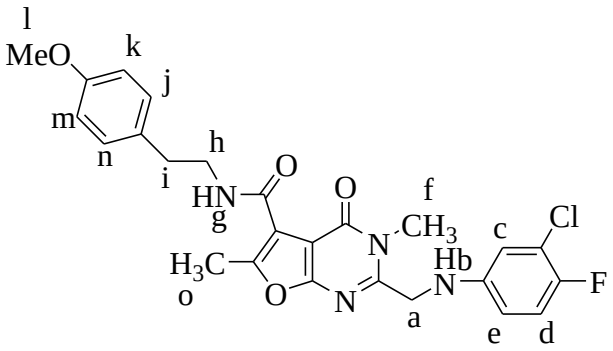


(264)

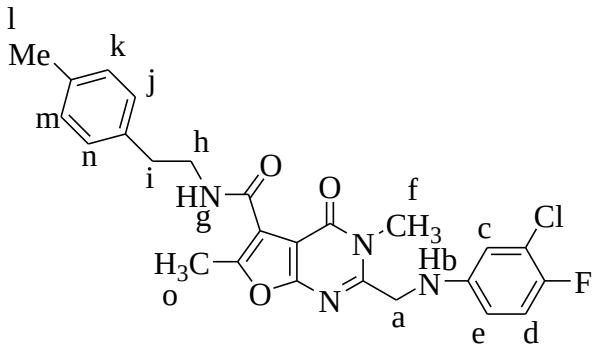
Compound (**264**) showed signals at 3385 and 3250 cm^{-1} for NH stretching and at 1674 and 1645 cm^{-1} for C=O stretching in its IR spectrum. The ^{1}H-NMR spectrum of the compound showed a triplet due to amidic proton at δ 10.0 (t, *J* = 5.5 Hz, 1H*g*, CON*H*) and four multiplets for seven aromatic protons at δ 7.26-7.24 (m, 2H*j, m*), 7.06-6.97 (m, 3H*k, l, d*), 6.77-6.75 (m, 1H*c*) and 6.64-6.61 (m, 1H*e*). A singlet for amino proton at δ 5.04 (s, 1H*b*), a doublet for two methylene attached to secondary amine (-NH*b*) at δ 4.38-4.37 (d, *J* = 4.0 Hz, 2H*a*, CH_2-NH) and a multiplet for five protons at δ 3.69-3.64 (m, 5H*f, h*, N-CH_3 & -CH_2-CH_2-NH) were visible. A triplet for two protons at δ 2.95 (t, *J* = 7.5 Hz, 2H*i*, CH_2-CH_2) and a singlet for methyl protons at δ 2.85 (s, 3H*n*, O-C-CH_3) too got displayed in aliphatic region. The HR-MS spectrum of the compound showed a peak at *m/z* 487.1343 $[M+H]^+$.

The IR spectrum of compound (**265**) exhibited peaks for NH stretching at 3376 and 3229 cm^{-1} and for C=O stretching at 1672 and 1646 cm^{-1}. The PMR spectrum of the compound exhibited peaks at δ 9.99 (t, *J* = 5.5 Hz, 1H*g*, CON*H*) as triplet for proton of amide and a doublet for two protons at δ 7.22-7.21 (d, *J* = 8.0 Hz, 2H*j, n*). A triplet for a proton at δ 7.04 (t, *J* = 8.5 Hz, 1H*d*) along with a doublet for two protons at δ 6.86-6.85 (d, *J* = 8.0 Hz, 2H*k*, *m*) and two multiplets for two protons at δ 6.76-6.75 (m, 1H*c*, Ar-*H*) and 6.63-6.60 (m, 1H*e*) were observed. A singlet at δ 5.06 (s, 1H*b*), a doublet at δ 4.37 (d, *J* = 4.0 Hz, 2H*a*, CH_2-NH) with a singlet for methoxy protons at δ 3.81 (s, 3H*l*) and a singlet for methyl protons at δ 3.70 (s, 3H*f*, N-CH_3) were observed in aliphatic area. Peaks at δ 3.65 (q, *J* = 6.5 Hz, 2H*h*, CH_2-CH_2-NH) as a quartet, δ

2.92 (t, *J* = 7.5 Hz, 2H*i*, C*H*$_2$-CH$_2$) as a triplet for four methylene protons and δ 2.84 (s, 3H*o*, O-C-C*H*$_3$) as a singlet were also noticed. The HR-MS spectrum of the compound offered *m/z* 499.1543 $[M+H]^+$.



(265)

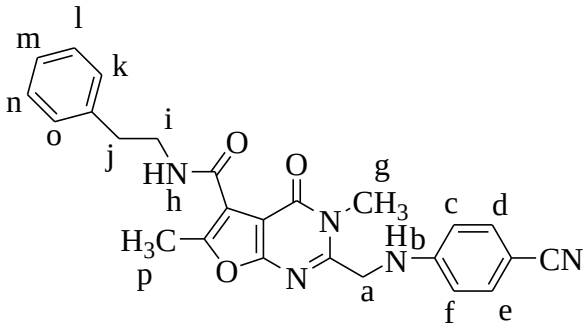


(266)

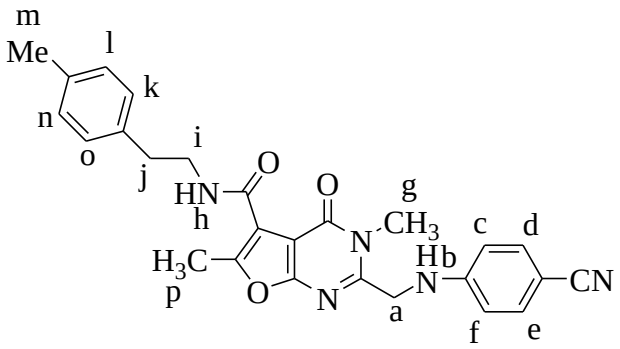
Signals for NH stretching were observed at 3385 and 3232 cm^{-1} and for C=O stretching at 1681 and 1636 cm^{-1} in the IR spectrum of compound (**266**). ^{1}H-NMR spectrum of the compound revealed signals at δ 10.00 (s, 1H*g*, CON*H*) as amidic proton and two doublets due to *ortho* coupling at δ 7.20-7.19 (d, *J* = 7.75 Hz, 2H*j*, *n*) and 7.13-7.12 (d, *J* = 7.75 Hz, 2H*k*, *m*) for four protons. A triplet at δ 7.04 (t, *J* = 9.0 Hz, 1H*d*) and two multiplets for two protons at δ 6.76-6.75 (m, 1H*c*, Ar-*H*) and 6.63-6.60 (m, 1H*e*) were afforded by other aromatic protons. Two singlets at δ 4.37 (s, 2H*a*, C*H*$_2$-NH), 3.70 (s, 3H*f*, N-C*H*$_3$) along with a multiplet at δ 3.68-3.64 (m, 2H*h*, CH$_2$-C*H*$_2$-NH) and a triplet at δ 2.94 (t, *J* = 8.0 Hz, 2H*i*, C*H*$_2$-CH$_2$) for four methylene protons, and two singlets for two methyl groups at δ 2.85 (s, 3H*o*, O-C-C*H*$_3$) and 2.34 (s, 3H*l*, Ar-C*H*$_3$) were displayed in the aliphatic region. ^{13}C-NMR spectrum of the compound afforded peaks at δ 162.05, 161.14, 160.24, 158.77, 154.12, 136.17, 135.73, 129.42, 129.14, 128.66, 117.09, 114.19, 112.81, 112.46, 102.99, 46.54, 41.15, 35.44, 29.78, 21.05 and 14.05. The mass spectrum of the compound was seen at *m/z* 483.3 $[M+H]^+$.

Compound (**267**) in its IR spectrum revealed characteristic peaks at 3037 cm^{-1} for NH stretching, 2223 cm^{-1} for CN stretching and 1668 and 1645 cm^{-1} for C=O stretching. The PMR spectrum of the compound afforded a triplet for the proton of amide at δ 9.96 (t, *J* = 5.5 Hz, 1H*h*, CON*H*), a doublet at δ 7.53-7.52 (d, *J* = 7.5 Hz, 2H*d, e*), and two multiplets for seven aromatic

protons at δ 7.19-7.11 (m, 5H*k, l, m, n, o*) and 6.76-6.74 (m, 2H*c, f*). Aliphatic protons showed a triplet at δ 5.76 (t, *J* = 4.5 Hz, 1H*b*), a doublet at δ 4.45 (d, *J* = 4.0 Hz, 2H*a*, C*H*$_2$-NH) and a singlet at δ 3.70 (s, 3H*g*, N-C*H*$_3$). A multiplet at δ 3.66-3.62 (m, 2H*i*, -CH$_2$-C*H*$_2$-NH) and a triplet at δ 2.93 (t, *J* = 7.5 Hz, 2H*j*, C*H*$_2$-CH$_2$) for four methylene protons were seen with a singlet for methyl group at δ 2.84 (s, 3H*p*, O-C-C*H*$_3$). ^{13}C-NMR spectrum of the compound exhibited carbon signals at δ 161.96, 160.96, 160.12, 158.87, 153.25, 149.59, 136.10, 135.76, 133.93, 129.14, 128.64, 119.96, 112.81, 112.45, 103.67, 100.46 and aliphatic carbons at δ 45.15, 41.16, 35.39, 29.77, 29.71, 21.05 and 14.06.



(267)

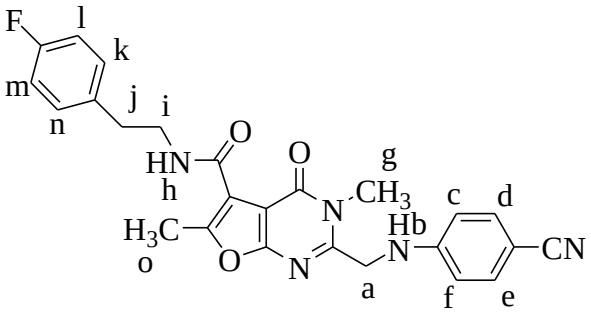


(268)

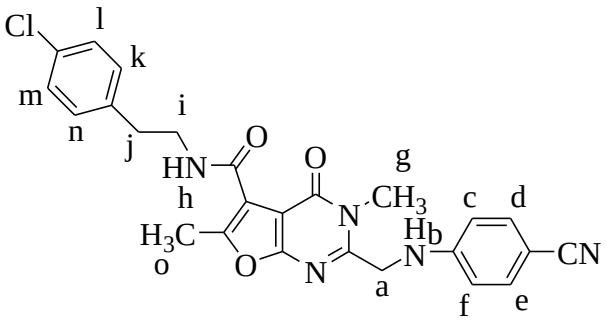
The IR spectrum of compound (**268**) exhibited signals for NH stretching at 3370 cm^{-1}, characteristic CN stretching at 2212 cm^{-1} and C=O stretching at 1675 cm^{-1}. ^{1}H-NMR spectrum of the compound showed two multiplets for aromatic protons at δ 7.55-7.42 (m, 4H*c, d, e, f*) and 6.86-6.66 (m, 4H*k, l, m, n*). Aliphatic region displayed a singlet at δ 5.74 (s, 1H*b*) and a doublet at δ 4.42-4.41 (d, *J* = 5.5 Hz, 2H*a*, C*H*$_2$-NH) along with a multiplet for two methylene protons at δ 3.84-3.78 (m, 2H*i*, CH$_2$-C*H*$_2$-NH) and a singlet at δ 3.67 (s, 3H*g*, N-CH$_3$). A multiplet for other methylene protons at δ 2.86-2.80 (m, *J* = 7.5 Hz, 2H*j*, C*H*$_2$-CH$_2$) and two singlets for two methyl groups at δ 2.70 (s, 3H*p*, O-C-C*H*$_3$) and δ 2.06 (s, 3H*m*, -C*H*$_3$) also appeared. The mass spectrum of the compound revealed quasimolecular ion peak at *m/z* 456.3 [M+H]$^+$.

Compound (**269**) displayed NH stretching at 3274 cm^{-1}, CN stretching at 2212 cm^{-1} and C=O stretching at 1666 cm^{-1} in its IR spectrum. PMR spectrum of the compound furmished a triplet at δ 9.95 (t, *J* = 5.5 Hz, 1Hh, CON*H*) for amide proton and four doublets each for two aromatic protons at δ 7.54-7.53 (d, *J* = 8.75 Hz, 2H*d, e*, Ar-*H*), 7.25-7.23 (d, *J* = 8.5 Hz, 2H*k, n*,

Ar-*H*), δ 6.99-6.97 (d, *J* = 8.5 Hz, 2H*l, m,* Ar-*H*) and 6.77-6.75 (d, *J* = 8.75 Hz, 2H*c, f,* Ar-*H*) while aliphatic region displayed a triplet for amine proton at δ 5.72 (t, *J* = 4.5 Hz, 1H*b*, CH_2-N*H*), a doublet for methylene at δ 4.46-4.45 (d, *J* = 4.0 Hz, 2Ha, C*H*$_2$-NH), a singlet for methyl linked to ring amide at δ 3.69 (s, 3H*g*, N-C*H*$_3$) and two peaks viz. a multiplet at δ 3.67-3.65 (m, 2H*i*, CH_2-C*H*$_2$-NH) and triplet at 2.95 (t, *J* = 7.5 Hz, 2H*j*, C*H*$_2$-CH_2) for ethylene protons along with a singlet at δ 2.84 (s, 3H*o*, O-C-C*H*$_3$) for the methyl group. Mass spectrum of the compound offered a peak at *m/z* 459.5 $[M]^+$.



(269)

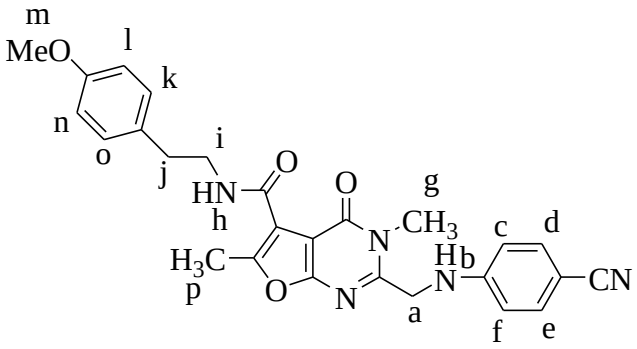


(270)

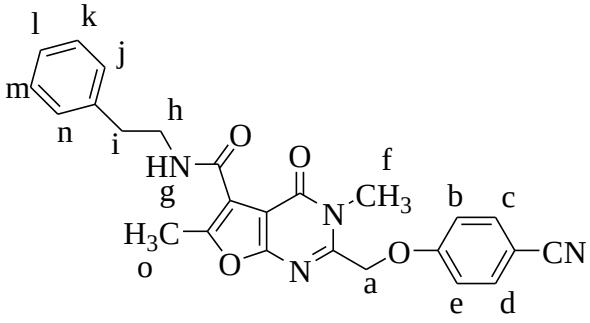
In the IR spectrum of compound (**270**) peaks were seen at 3078 and 2924 cm^{-1} for NH stretching, 2216 cm^{-1} for CN stretching and at 1676 and 1649 $cm^{-1}$for C=O stretching. In its PMR spectrum, the compound offered amidic proton triplet at δ 9.95 (t, *J* = 5.5 Hz, 1H*h*, CON*H*) and three doublets corresponding to six protons at δ 7.55-7.53 (d, J = 9.0 Hz, 2H*d, e*), 6.99-6.97 (d, *J* = 9.0 Hz, 2H*l, m*) and 6.77-6.75 (d, *J* = 9.0 Hz, 2H*c, f*) with a multiplet for two protons at 7.24-7.22 (m, 2H*k, n*). A singlet at δ 4.92 (s, 1H*b*, CH_2-N*H*) for amine proton and a doublet at δ 4.44-4.43 (d, *J* = 7.0 Hz, 2H*a*, C*H*$_2$-NH) for two methylene protons were also observed. Other aliphatic protons gave a multiplet for five protons at δ 3.68-3.65 (m, 5H*g, i*, -CH_2-C*H*$_2$-NH & -N-C*H*$_3$), a triplet for methylene at δ 2.95 (t, *J* = 7.5 Hz, 2H*j*, C*H*$_2$-CH_2) and singlet for methyl at δ 2.85 (s, 3H*o*, O-C-C*H*$_3$). The HR-MS spectrum of the compound showed *m/z* 476.1484 $[M+H]^+$.

Compound (**271**) showed signals for NH stretching at 3391 and 3247 cm^{-1}, for nitrile CN stretching at 2214 cm^{-1} and C=O stretching at 1677 and 1649 cm^{-1} in its IR spectra. The PMR spectrum of the compound exhibited a triplet at δ 9.95 (t, *J* = 5.5 Hz, 1H*h*, CON*H*) and four doublets due to *ortho* coupling in aromatic protons at δ 7.54-7.53 (d, *J* = 8.5 Hz, 2H*d, e*), 7.23-

7.21 (d, *J* = 8.25 Hz, 2H*k, o*), 6.86-6.85 (d, *J* = 8.25 Hz, 2H*l, n*) and 6.77-6.75 (d, *J* = 8.5 Hz, 2H*c, f*). A triplet for secondary amine proton at δ 5.72 (t, *J* = 4.5 Hz, 1H*b*, CH_2-N*H*), a doublet for amino-methylene at δ 4.46-4.45 (d, *J* = 4.5 Hz, 2H*a*, CH_2-NH), two singlets each for methoxy and methyl groups at δ 3.81 (s, 3H*m*, OCH_3) and 3.70 (s, 3H*g*, -N-CH_3), a quartet and a triplet for methylene protons at δ 3.65 (q, *J* = 7.0 Hz, 2H*i*, CH_2-CH_2-NH) and δ 2.92 (t, *J* = 7.5 Hz, 2H*j*, CH_2-CH_2) and a singlet at δ 2.85 (s, 3H*p*, O-C-CH_3) for methyl protons were also present. The HR-MS of the compound offered $[M+H]^+$ peak at *m/z* 472.1979.



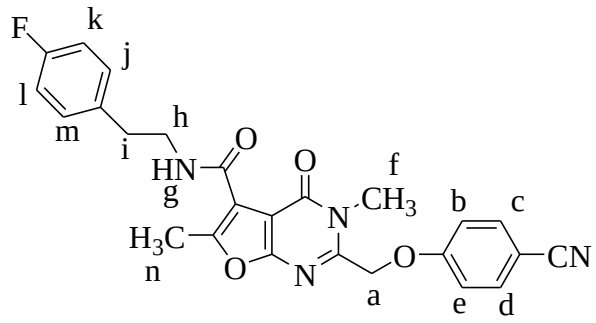
(271)



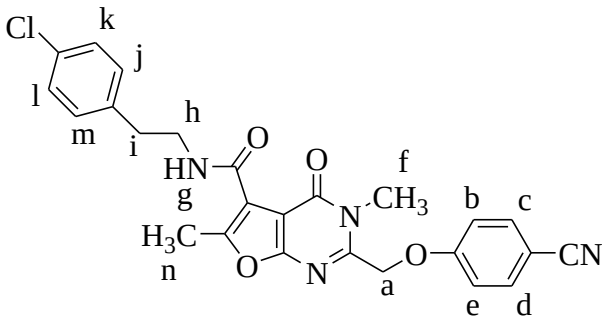
(272)

The IR spectrum of compound (**272**) offered signals at 3060 cm^{-1} for NH stretching, 2222 cm^{-1} for CN stretching and 1672 cm^{-1} for carbonyl stretching. PMR spectrum of the compound displayed signals at δ 10.01 (s, 1H*g*, CON*H*) as triplet for the amide proton and at δ 7.66-7.65 (d, *J* = 9.0 Hz, 2H*c, d*) as doublet for two protons. Aromatic protons showed two multiplets at δ 7.32-7.29 (m, 4H*j, k, m, n*) and 7.24-7.21 (m, 1H*l*) in addition to a doublet at δ 7.16-7.14 (d, *J* = 9.0 Hz, 2H*b, e*). Two singlets at δ 5.28 (s, 2Ha, CH_2-O) and 3.78 (s, 3H*f*, -N-CH_3), a multiplet at δ 3.71-3.66 (m, 2H*h*, CH_2-CH_2-NH), a triplet at δ 2.97 (t, *J* = 8.0 Hz, 2H*i*, CH_2-CH_2) and a singlet at δ 2.86 (s, 3H*o*, O-C-CH_3) were afforded by aliphatic protons. ^{13}C-NMR of the compound displayed signals for carbons at δ 161.54, 160.81, 160.39, 159.68, 151.44, 139.25, 134.34, 128.80, 128.44, 126.27, 115.67, 112.62, 106.03, 104.52 and for aliphatic carbons at δ 69.24, 40.99, 35.87, 31.57 and 14.15. The mass spectrum of the compound revealed $[M+H]^+$ peak at *m/z* 443.3.

In the IR spectrum of compound (**273**) peaks were observed at 3230 and 3044 cm^{-1} for NH stretching, 2225 cm^{-1} for CN stretching and 1676 cm^{-1} for C=O stretching. Proton NMR spectrum of the compound afforded a singlet for amide linked proton at δ 9.96 (s, 1H*g*, CON*H*) and a doublet for two protons at δ 7.64-7.62 (d, *J* = 8.8 Hz, 2H*c*, *d*). A multiplet at δ 7.24-7.21 (m, 2H*j*, *m*), a doublet at δ 7.14-7.12 (d, *J* = 8.8 Hz, 2H*b*, *e*) and a triplet at 6.98-6.96 (d, *J* = 8.8 Hz, 2H*k*, *l*) all accounting for two protons each were noticed. Two singlets were seen at δ 5.25 (s, 2H*a*, -CH_2-O-) and 3.75 (s, 3H*f*, -N-CH_3) along with a quartet at δ 3.66-3.64 (q, *J* = 7.4 Hz, 2H*h*, CH_2-CH_2-NH) and a triplet at δ 2.91 (t, *J* = 8.0 Hz, 2H*i*, CH_2-CH_2) for four methylene protons. A methyl group furnished a singlet at δ 2.83 (s, 3H*n*, O-C-CH_3). The HR-MS of the compound offered a peak at *m/z* 461.1620 $[M+H]^+$.



(**273)**

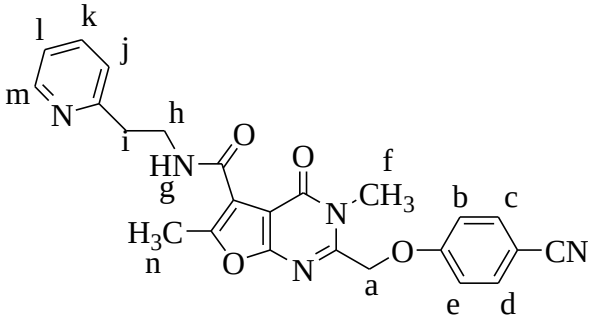


(274)

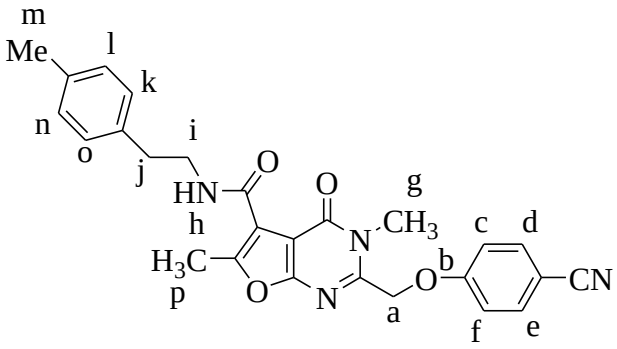
Compound (**274**) in its IR spectrum exhibited peaks for NH stretching at 3480 cm^{-1}, for CN stretching at 2226 cm^{-1} and for C=O stretching at 1677 and 1642 cm^{-1}. PMR spectrum of the compound displayed a broad singlet at δ 9.99 (bs, 1H*g*, -CON*H*) for the amidic proton and four multiplets accounting for eight protons at δ 7.25-7.22 (m, 2H*c*, *d*), 7.20-7.18 (m, 2H*k*, *l*), δ 6.96-6.92 (m, 2H*j*, *m*) and 6.70-6.66 (m, 2H*b*, *e*). Two singlets for methylene and methyl protons at δ 4.36 (s, 2H*a*, CH_2-O-) and 3.66 (s, 3H*f*, -N-CH_3) respectively were revealed along with a quartet at δ 3.65-3.63 (q, *J* = 7.3 Hz, 2H*h*, CH_2-CH_2-NH) and triplet at δ 2.91 (t, *J* = 7.8 Hz, 2H*i*, CH_2-CH_2) for the amide-linked ethylene protons and a singlet at δ 2.80 (s, 3H*n*, O-C-CH_3).

Compound (**275**) displayed signals at 3237 and 3059 cm^{-1} for NH stretching, characteristic CN stretching at 2221 cm^{-1} and amide C=O at 1669 cm^{-1} in its IR spectra. PMR spectrum of compound offered a triplet at δ 10.01 (t, *J* = 5.2 Hz, 1H*g*, CON*H*) for proton of the

amide. Aromatic protons displayed three multiplets at δ 8.56-8.54 (m, 1H*m*), 7.64-7.60 (m, 3Hc, d, k) and 7.14-7.11 (m, 3H*b, e, j*) and one doublet at δ 7.25-7.23 (d, *J* = 7.8 Hz, 1H*l*). A singlet at δ 5.25 (s, 2H*a*, C*H*$_2$-O-) for methylene and a quartet at δ 3.84-3.81 (q, *J* = 7.0 Hz, 2H*h*, CH$_2$-C*H*$_2$-NH) for another methylene were obtained in the aliphatic region. A singlet for methyl of tertiary amide at δ 3.73 (s, 3H*f*, -N-C*H*$_3$), a triplet for methylene linker at δ 3.13 (t, *J* = 7.5 Hz, 2H*i*, C*H*$_2$-CH$_2$) and a singlet at δ 2.81 (s, 3H*n*, O-C-C*H*$_3$) also appeared. ^{13}C-NMR spectrum of the compound exhibited peaks for carbons at δ 161.99, 160.75, 160.38, 160.24, 159.57, 159.27, 151.45, 149.39, 136.33, 134.32, 123.27, 121.36, 118.57, 115.67, 112.61, 105.98, 104.47 and at δ 69.22, 39.19, 38.01, 31.41, 29.70 and 14.13. The mass spectrum of the compound gave a peak at *m/z* 444.3 $[M+H]^+$.



(275)

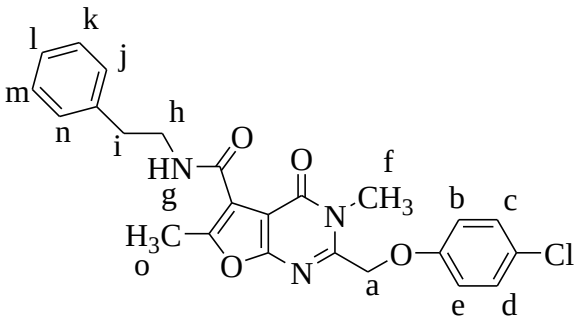


(276)

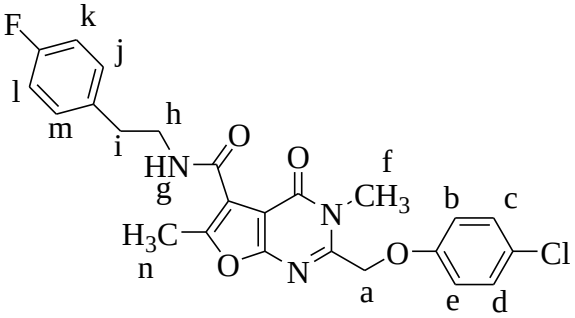
Compound (**276**) in its IR spectrum showed peaks for NH stretching at 3053 cm^{-1}, nitrile at 2224 cm^{-1} and C=O at 1672 cm^{-1}. PMR spectra of compound exhibited signals at δ 9.96 (t, *J* = 5.2 Hz, 1H*h*, CON*H*) as a triplet of amide proton and δ 7.65-7.64 (d, *J* = 8.8 Hz, 2H*d*, *e*) as doublet for two protons. A multiplet for six aromatic protons was also seen at δ 7.18-7.08 (m, 6H*c, f, k, l, n, o*). Two singlets representing methylene and methyl protons were observed at δ 5.25 (s, 2H*a*, C*H*$_2$-O-) and 3.75 (s, 3H*g*, N-C*H*$_3$). Four methylene protons offered a multiplet at δ 3.66-3.63 (m, 2H*i*, -CH$_2$-C*H*$_2$-NH) and a triplet at δ 2.91 (t, *J* = 8.0 Hz, 2H*j*, C*H*$_2$-CH$_2$). Two singlets corresponding to two methyl groups were displayed at δ 2.83 (s, 3H*p*, O-C-C*H*$_3$) and 2.31 (s, 3H*m*).

The IR spectrum of compound (**277**) displayed signals at 3233 and 3059 cm^{-1} for NH stretching, and 1673 and 1649 cm^{-1} for C=O stretching. Compound (**277**) in its ^{1}H-NMR

spectrum revealed a triplet at δ 10.06 (t, *J* = 5.5 Hz, 1H*g*, CON*H*) for the proton of the amide and two multiplets for six aromatic protons at δ 7.31-7.28 (m, 6H*c, d, j, k, m, n*, Ar-*H*) and one proton at δ 7.25-7.21 (m, 1H*l*). A doublet due to *ortho* coupling was also seen at δ 7.00-6.98 (d, *J* = 9.0 Hz, 2H*b, e*). Aliphatic protons displayed two singlets for methylene and methyl protons at δ 5.19 (s, 2H*a*, C*H*$_2$-O-) and 3.78 (s, 3H*f*, N-C*H*$_3$) respectively with a multiplet at δ 3.70-3.66 (m, 2Hh, CH$_2$-C*H*$_2$-NH) and triplet at δ 2.98 (t, *J* = 8.0 Hz, 2H*i*, C*H*$_2$-CH$_2$) for ethylenes and a singlet at δ 2.85 (s, 3H*o*, O-C-C*H*$_3$). ^{13}C-NMR spectrum of the compound showed signals for carbons at δ 162.04, 160.96, 160.50, 159.46, 155.82, 152.27, 139.29, 129.77, 128.81, 128.44, 127.49, 126.25, 116.23, 112.57, 104.31 and at δ 69.53, 41.00, 35.90, 31.54, 14.13 for aliphatic carbons. The HR-MS spectrum of the compound exhibited *m/z* 452.1372 [M+H]$^+$



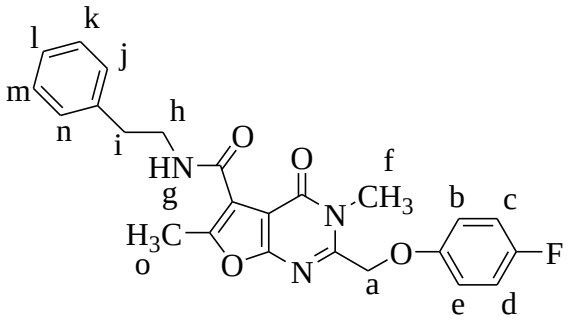
(277)



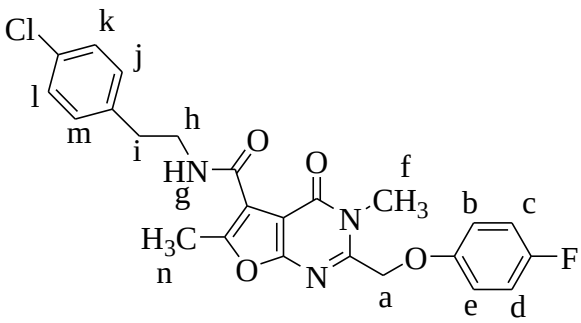
(278)

Peaks for NH stretching at 3229 and 3038 cm^{-1} and C=O stretching at 1680 and 1640 cm^{-1} were seen in IR spectrum of compound (**278**). The PMR spectrum of compound (**278**) showed a triplet at δ 10.04 (t, *J* = 4.8 Hz, 1H*g*, CON*H*) along with two multiplets at δ 7.31-7.29 (m, *2Hc, d*) and 7.26-7.23 (m, 2H*j*, *m*) for four aromatic protons, and another multipet comprising of four protons at δ 7.01-6.97 (m, 4H*b, e, k, l*). Two singlets for five protons at δ 5.19 (s, 2H*a*, C*H*$_2$-O-) and 3.78 (s, 3H*f*, N-C*H*$_3$) and peaks for ethylene protons appeared as a multiplet at δ 3.68-3.64 (m, 2H*h*, CH$_2$-C*H*$_2$-NH) and a triplet at δ 2.94 (t, *J* = 6.0 Hz, 2H*i*, C*H*$_2$-CH$_2$) with a singlet for methyl protons at δ 2.85 (s, 3H*n*, O-C-C*H*$_3$). Carbon NMR spectrum offered peaks at δ 162.06, 160.97, 160.50, 159.51, 155.82, 152.29, 139.29, 130.23, 130.17, 129.78, 127.51, 116.23, 115.27, 115.11, 104.28 and at δ 69.53, 40.97, 35.01, 31.54, 14.12 for aliphatic carbons. The mass spectrum of the compound offered m/z 470.3 [M+H]$^+$.

The IR spectrum of compound (**279**) exhibited peaks of NH stretching at 3228 and 3046 cm^{-1} and amide C=O stretching at 1677 and 1640 cm^{-1}. The PMR spectrum of the compound offered a singlet at δ 10.04 (s, 1H*g*, CON*H*) for the proton of amide and three multiplets at δ 7.31-7.26 (m, 4H*j, k, m, n*) for four protons, δ 7.23-7.18 (m, 1H*l*) for one proton and δ 7.03-6.95 (m, 4H*b, c, d, e*) for other four aromatic protons. Two singlets for methylene protons at δ 5.15 (s, 2H*a*, -C*H*$_2$-O-) and for tertiary amido methyl at δ 3.77 (s, 3H*f*, -N-C*H*$_3$) were noticed in addition to a multiplet at δ 3.69-3.63 (m, 2H*h*, -CH$_2$-C*H*$_2$-NH) and triplet at δ 2.96 (t, *J* = 8.0 Hz, 2H*i*, C*H*$_2$-CH$_2$) for ethylene protons with a singlet for three methyl protons at δ 2.82 (s, 3Ho, O-C-C*H*$_3$).



(279)

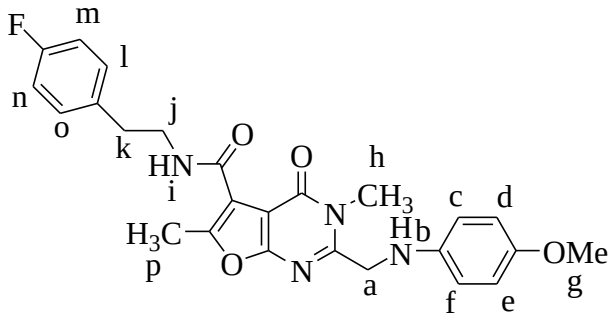


(280)

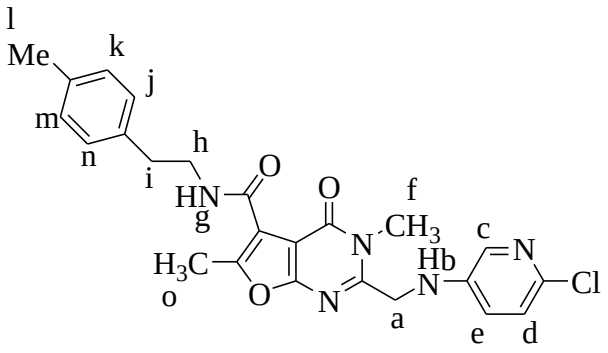
Compound (**280**) afforded IR signals at 3457 and 3253 cm^{-1} for NH stretching along with C=O stretching at 1678 and 1640 cm^{-1}. ^{1}H-NMR spectrum of the compound furnished a peak at δ 10.02 (s, 1H*g*, CON*H*) as a singlet and two multiplets for eight aromatic protons at δ 7.24-7.19 (m, 4H*j, k, l, n*) and 7.03-6.95 (m, 4H*b, c, d, e*). Aliphatic protons exhibited two singlets at δ 5.15 (s, 2Ha, C*H*$_2$-O-) and 3.76 (s, 3H*f*, N-C*H*$_3$). A quartet and triplet both for ethylene linker were observed at δ 3.66-3.64 (q, *J* = 7.2 Hz, 2H*h*, CH$_2$-C*H*$_2$-NH) and 2.92 (t, *J* = 7.4 Hz, 2H*i*, C*H*$_2$-CH$_2$) respectively along with a singlet for methyl protons at δ 2.82 (s, 3H*n*, O-C-C*H*$_3$). The HR-MS spectrum of the compound exhibited a peak at *m/z* 470.1277 $[M+H]^+$.

The IR spectrum of compound (**281**) offered peaks at 3391 and 3259 cm^{-1} for NH stretching and C=O stretching at 1669 cm^{-1}. It's ^{1}H-NMR afforded a triplet of amidic proton at δ 10.05 (t, *J* = 4.8 Hz, 1H*i*, CON*H*), and four multiplets representing eight aromatic protons at δ 7.26-7.23 (m, 2H*l, o*), 7.01-6.97 (m, 2H*m, n*), 6.87-6.84 (m, 2H*d, e*) and δ 6.77-6.74 (m, 2H*c, f*).

For aliphatic protons signals were observed as three singlets at δ 4.41 (s, 2Hb, C*H*$_2$-NH), 3.78 (s, 3Hg, -OCH$_3$) and 3.70 (s, 3H*h*, N-C*H*$_3$) in addition to a multiplet at δ 3.68-3.64 (m, 2H*j*, CH$_2$-C*H*$_2$-NH), a triplet at δ 2.95 (t, *J* = 6.4 Hz, 2H*k*, C*H*$_2$-CH$_2$) for methylene protons and a singlet for methyl protons at δ 2.84 (s, 3Hp, Ar-C*H*$_3$).



(281)



(282)

Compound (**282**) revealed IR signals for NH stretching at 3417 and 3263 cm^{-1}, and for C=O stretching at 1667 cm^{-1}. Its PMR spectrum exhibited a triplet at δ 10.06 (t, *J* = 5.5 Hz, 1H*g*, CON*H*) and a doublet for one pyridyl proton at δ 8.08-8.07 (d, *J* = 2.5 Hz, 1H*c*). A doublet of doublet for another proton of pyridine moiety was observed at δ 7.44-7.43 (dd, *J* = 2.5 & 7.5 Hz, 1H*e*). Two multiplets at δ 7.22-7.19 (m, 2H*j*, *n*) and 7.14-7.11 (m, 2H*k*, *m*) for aromatic protons were also observed with a doublet at δ 6.63-6.61 (d, *J* = 9.0 Hz, 1Hd). Aliphatic region displayed a doublet at δ 4.72 (d, *J* = 4.5 Hz, 2H*a*, C*H*$_2$-NH), singlet at δ 3.72 (s, 3H*f*, N-C*H*$_3$), two multiplets for methylene protons at δ 3.68-3.64 (m, 2H*h*, CH$_2$-C*H*$_2$-NH) and 2.94 (m, 2H*i*, C*H*$_2$-CH$_2$), and two singlets for methyl groups at δ 2.82 (s, 3H*o*, Ar-C-C*H*$_3$) and 2.34 (s, 3H*l*). The mass spectrum of the compound gave quasimolecular ion peak at *m/z* 466.3.

4.2.2 Biological Evaluation

4.2.2.1 *In vitro* FIIa (thrombin) enzyme inhibition study

The synthesized target compounds were biologically evaluated for the antithrombotic activity by *in vitro* thrombin (FIIa) enzyme inhibition assay[4] taking dabigatran as a standard. The results revealed that all of these compounds showed more than 50% inhibition at 20 µM concentration (**Figure 4.6-4.7**). Those compounds that exhibited > 60% inhibition (total 22

compounds) from enzyme assay were selected for their IC_{50} values' determination. From the results it was observed that several compounds from the series displayed good inhibitory activities against thrombin enzyme with IC_{50} values from 0.96 to 68.6 µM (**Table 4.8**). Compounds (**255** and **259**) containing 4-chloro and 4-fluorophenyl substituents as S1-binding fragments were found to be the most potent compounds having IC_{50} values of 0.96 and 1.36 µM respectively.

To check the selectivity of the inhibitors for thrombin, a FXa specific chromogenic assay was also performed in which negligible inhibition (< 10%) against FXa enzyme was observed for all of the compounds.

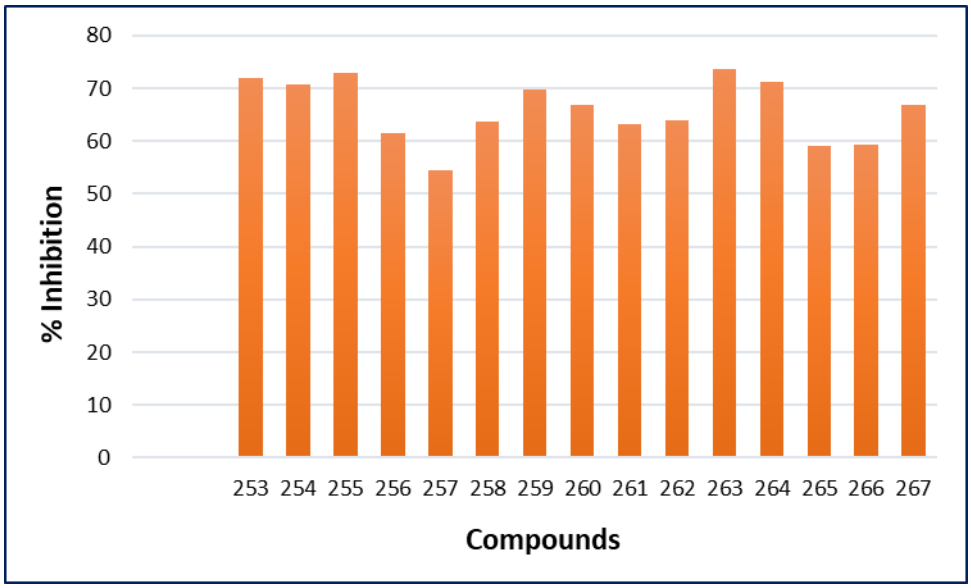


Figure 4.6: *In vitro* FIIa inhibition activity of furanopyrimidinone derivatives (**253-267**)

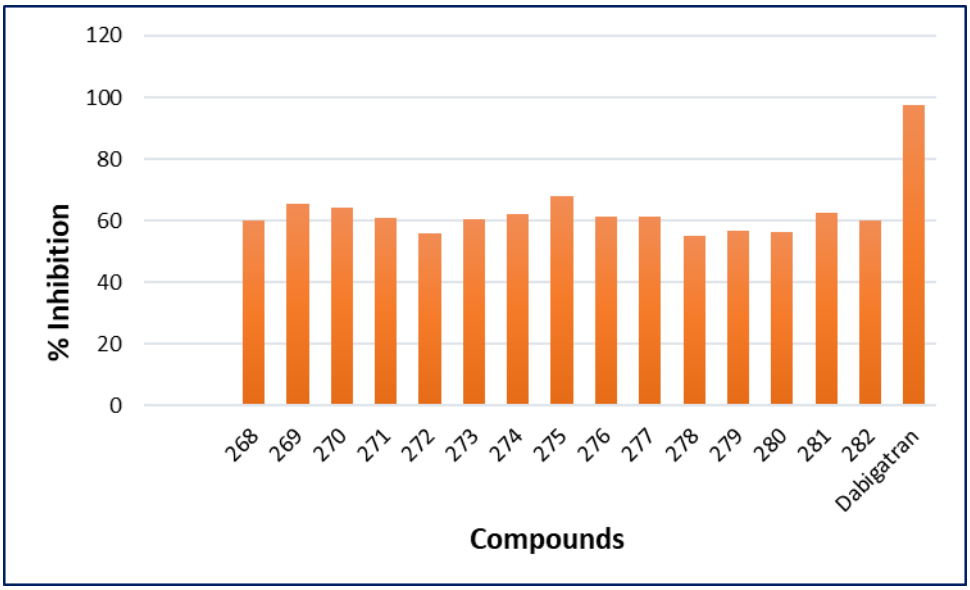


Figure 4.7: *In vitro* FIIa inhibition activity of furanopyrimidinone derivatives (**268-282**).

Table 4.8: IC_{50} values of compounds for FIIa inhibitory activity.

Compound	FIIa IC_{50} (µM)	Compound	FIIa IC_{50} (µM)
253	9.48 ± 1.4	**269**	31.2 ± 5.6
254	1.43 ± 0.3	**270**	23.6 ± 3.7
255	**0.96 ± 0.2**	**271**	52.2 ± 6.3
256	21.7 ± 4.8	**273**	47.3 ± 4.4
258	6.53 ± 1.3	**274**	38.7 ± 3.6
259	1.36 ± 0.4	**275**	25.4 ± 3.8
260	25.2 ± 4.7	**276**	42.6 ± 5.2
261	18.8 ± 2.6	**277**	55.4 ± 7.8
262	8.69 ± 1.2	**281**	68.6 ± 8.1
263	3.01 ± 0.7	**282**	21.4 ± 4.3
264	4.55 ± 0.8	**Dabigatran**	0.058 ± 0.006
267	34.6 ± 9.3	-	-

The results are expressed in mean ± SEM (n = 3).

Compounds (**255** and **259**) having 4-chloro and 4-fluorophenyls as S1-binding motifs and 4-chloro- and 4-fluorophenethyls as S3-binding ligands exhibited the most potent inhibition activity (**255,** IC_{50} = 0.96 µM; **259,** IC_{50} = 1.36 µM). Removal or replacement of these halogen substituents by electron-donating groups like methyl or methoxy on S1-binding ligands resulted in compounds (**256, 260, 261** and **282**) with significant loss in activity. 3-Chloro-4-fluorophenyl P1 fragments containing compounds (**263,** IC_{50} = 3.01 µM; **264,** IC_{50} = 4.55 µM) also provided moderate inhibitory activity while 4-cyanophenyl compounds (**267**; IC_{50} = 34.6 µM, **269**; IC_{50} = 31.2 µM; **270**; IC_{50} = 23.6 µM, **271**; IC_{50} = 52.2 µM) were found to show low potency. For S1-binding substituted anilino derivatives biological activity ranges in descending order as 4-chloro > 4-fluoro > 3-chloro-4-fluoro > 4-cyano > 4-methoxy. Substituting phenyl ring with 5-chloro-2-pyridyl as P1 moiety gave compound (**282,** IC_{50} = 21.4 µM) having reduced potency. Further optimization of S1-binding fractions in the form of phenolic moieties did not improve the enzyme inhibition activity (**273**; IC_{50} = 47.3 µM; **274**; IC_{50} = 38.7 µM, **277**; IC_{50} = 55.4 µM).

p-Halo-substituted phenyl P3 ligand containing compounds were found to be the most potent derivatives (compounds (**255**, **259**, **263**, **264**). Methyl or methoxy substituents on phenyl ring offered compounds having decreased activity (e.g. **256, 260, 261, 271, 276**). Replacement of phenyl moiety by 2-pyridyl ring as S3-binding fragment and 4-haloanilino as S1-ligand displayed good activity (**254**; IC_{50} = 1.43 µM) but the activity decreased for compounds with S3 fragments like 4-cyanophenyl (**275**; IC_{50} = 25.4 µM).

From the assessment of PT and aPTT determination, the most potent compound (**255,** PT**=** 18.7 and aPTT= 33.4 sec.) was found to be less active than standard drug dabigatran but it showed better prolongation than control vehicle (8.7 and 25.3 sec.) while compounds like (**258**) and (**264**) displayed comparable activity to the control. In the evaluation of bleeding tendency, compound (**255**) showed lesser bleeding time (BT= **91** seconds) compared to the standard dabigatran (BT= 102 seconds) signifying its safety profile.

4.2.2.2 *Ex vivo* prothrombin time (PT) and activated partial thromboplastin time (aPTT) prolongation and bleeding time in rats

The synthesized compounds exhibiting potent biological activity from enzyme inhibition studies were evaluated for their anticoagulation potential by *ex vivo* prothrombin time (PT) and

activated partial thromboplastin time (aPTT) prolongation in rats.[5] PT indicates the influence of the inhibitor on extrinsic pathway of coagulation while aPTT represents its effect on intrinsic pathway of coagulation.[27] The activity was determined in rats after 2 hrs of oral administration of the inhibitor compounds at a dose of 30 mg/kg. The results (**Table 4.9**) demonstrated that compounds (**255**; 18.7 and 33.4 sec. and **264**; 11.7 and 35.3 sec.) showed higher prolongation in both PT and aPTT compared to the control (8.7 and 25.3 sec) but less than the standard drug dabigatran (26.1 and 101 sec.).

The hemorrhagic property caused by the target compounds was evaluated by bleeding time measurement.[6] The results (**Table 4.9**) revealed that bleeding times were not significantly higher at the antithrombotic doses except for compounds (**256** and **262**). Interestingly compound (**255**) has exhibited low bleeding time (BT= 91 sec.) compared to the standard drug dabigatran (102 sec.).

Table 4.9: *Ex vivo* PT and aPTT prolongation and bleeding time in rats

Compound	**PT (sec.)**	**aPTT (sec.)**	**Bleeding time (sec**.) at 30 mg/kg dose
253	10.6	27.5	122
254	11.5	18.9	145
255	**18.7**	**33.4**	**91**
256	9.2	19.2	156
258	**11.5**	**29.9**	106
259	9.9	18.5	135
261	10.3	22.3	128
262	9.8	20.6	165
263	**13.2**	27.4	136
264	11.7	**35.3**	124
Dabigatran	**26.1**	**101**	**102**
Control	8.7	25.3	78

4.2.3 Molecular Docking Studies

To examine the molecular interactions of the synthesized compounds with the thrombin enzyme molecular docking was carried out. The docking scores of active compounds from the series are displayed in **Table 4.10**. Compound (**259**) exhibited promising enzyme interactions with the highest docking score of -9.35 signifying high affinity nearly similar to the standard drug dabigatran. The overlaid structures of the most active compound (**255**) biologically and the standard drug dabigatran in **Figure 4.9** indicated similar binding interactions with thrombin enzyme. The molecular binding of compound (**255**) with thrombin enzyme is showed in **Figure 4.8**. 4-Chlorophenyl moiety showed hydrophobic bonding and electrostatic interactions with Asp189 residue of S1 binding site. The central pyrimidinone scaffold formed pi-pi stacking interactions with the Tyr60A-Trp60D pocket of S2 binding site which is essential for thrombin activity. Methyl substitution on the cyclic amide nitrogen provided conformational constraint for S2 binding and selectivity for thrombin over trypsin. The bonding of side chain amide carbonyl with Tyr 60A of S2 site and methylene side chain seems to be a non-classical hydrogen bonding. The terminal 4-fluorophenyl moiety is placed between the Leu99 and Ile174 residues of lipophilic distal S3 binding site where 4-fluoro group formed hydrophobic interactions with Asn98 residue of the enzyme. Other important van der Waals interactions were also observed between the compound and important residues of the enzyme e.g. Glu97A, Gly216, Glu192, Ser214, Gly219, Ala190 and Gly226

Table 4.10: Molecular Docking scores of some selected target compounds

Compound	Docking score
254	-7.34
255	-8.49
258	-8.07
259	-9.35
262	-7.76
263	-7.59
264	-8.34
275	-7.15
Dabigatran	-9.33

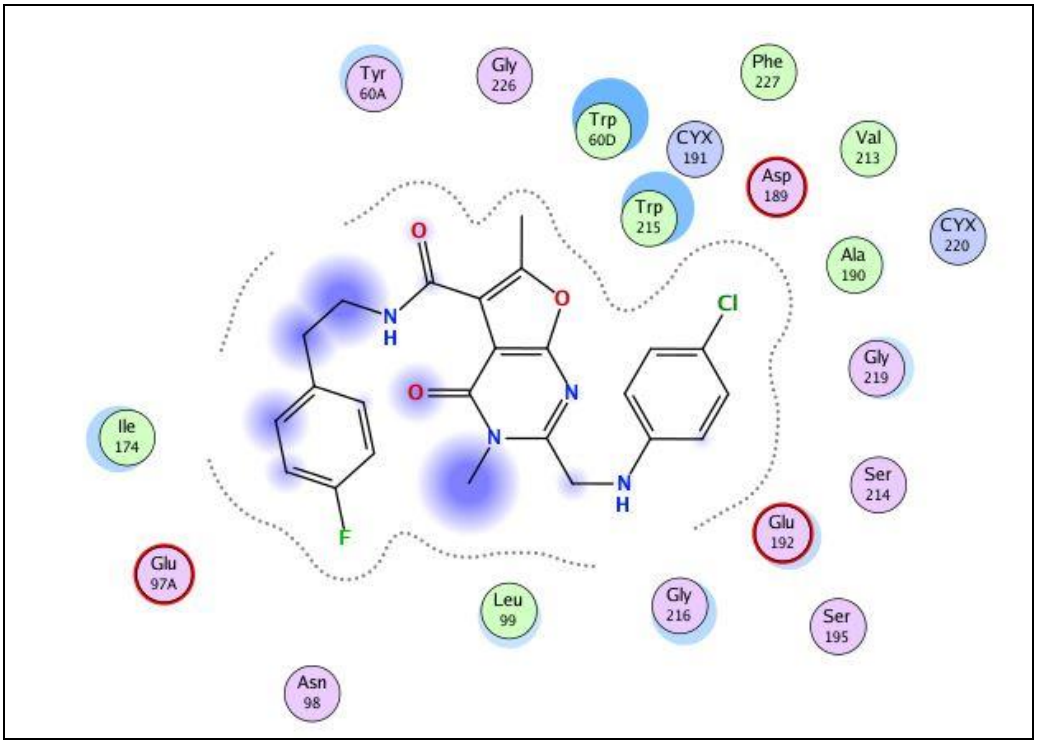


Figure 4.8: 2D interaction pose for compound (**255**) with thrombin enzyme.

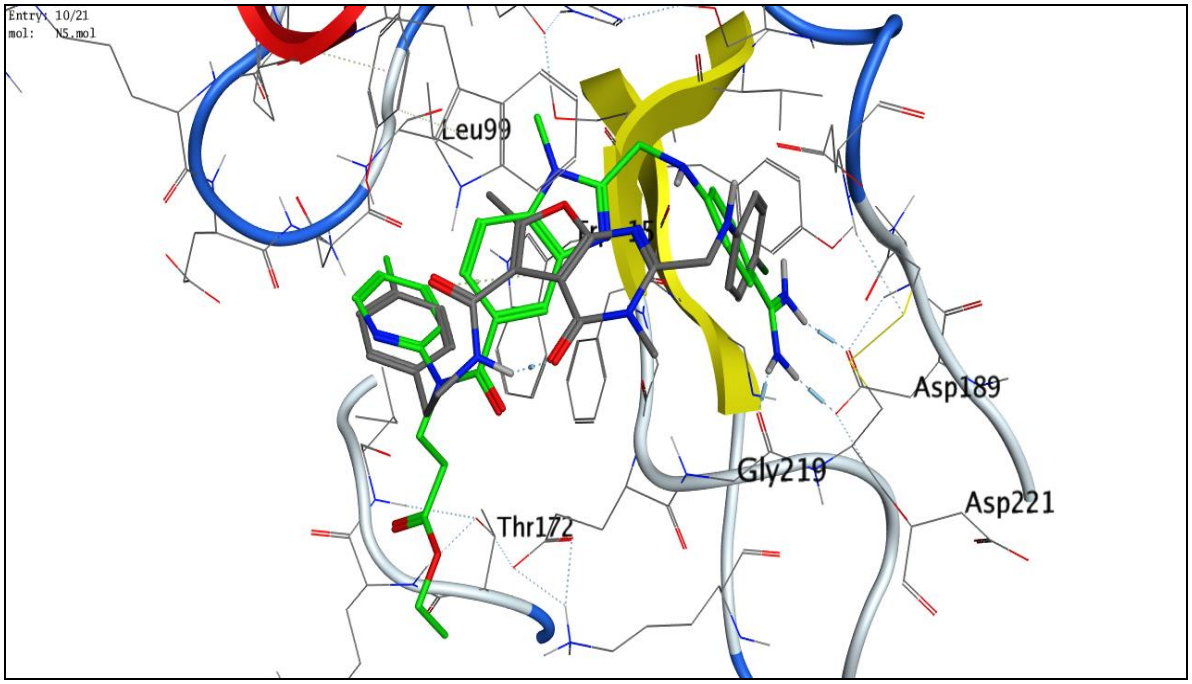


Figure 4.9: Overlaid structure of compound (**255**) and dabigatran with thrombin enzyme.

4.2.4 Prediction of Physicochemical and Pharmacokinetic Properties

Determination of physicochemical and pharmacokinetic properties of the lead compounds is considered as one of the key aspects in drug discovery and development field. The ADMET parameters viz. absorption, distribution, metabolism, excretion, toxicity as well as drug-likeliness and pharmacokinetic properties of synthesized compounds were investigated by freely available web servers SwissADME and pkCSM.[14] The results for these parameters are displayed in **Table 4.11** where all the compounds exhibited drug-likeliness properties within the standard ranges of Lipinski's rule of five without any violation. The intestinal absorption for all compounds was found to be fair with > 90% signifying their oral usefulness. Also none of the synthesized compounds displayed blood-brain barrier permeability indicating no CNS toxicity. The excretion and toxicity values were also found to be favourable for drug-like properties with total clearance in acceptable range and no hERG inhibition and AMES toxicity. Together, all these findings clearly suggested that the synthesized compounds have good lead potential for their development as orally active antithrombotic agents.

Table 4.11: Calculated physicochemical and pharmacokinetic properties of the synthesized compounds and Dabigatran

Compound	Mol. Wt	No. of HBD	No. of HBA	Log P	TPSA A^2	No. of rotatable bonds	Caco2 permeability	% Intestinal Absorption (human)	BBB permeability	Metabolism by
Dabigatran	471.51	4	6	1.79	150.22	10	-0.83	58.73	No	CYP3A4
254	451.91	2	5	3.31	102.05	8	1.20	93.52	No	CYP3A4
255	468.91	2	5	3.37	89.16	8	1.07	91.49	No	CYP3A4
258	434.46	2	5	3.34	89.16	8	1.045	92.91	No	CYP3A4
259	468.91	2	5	3.81	89.16	8	0.678	91.25	No	CYP3A4
263	503.35	2	5	3.72	89.16	8	1.17	92.14	No	CYP3A4
264	486.90	2	6	3.65	89.16	8	1.38	94.97	No	CYP3A4
275	443.45	1	7	3.25	123.04	8	0.53	95.24	No	CYP3A4

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